

THE AMERICAN NATURALIST

VOL. LXXIV *January-February, 1940*

No. 750

THE CELL THEORY. II*

A MODERN CONCEPT OF THE CELL AS A STRUCTURAL UNIT¹

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YEAR by year, step by step, since Wöhler synthesized urea in the laboratory in 1828 and showed that organic chemistry, the chemistry of life, was merely a more complicated inorganic chemistry; that the difference between the compounds formed in the living organism and those outside the living world was not one of *kind* but merely of *degree of complexity*, the boundary wall between the chemistry of life and non-life has been repeatedly shattered. It is now becoming increasingly apparent that the plan of organization exhibited in the living world, with the cell as the basic unit, does not differ intrinsically from that exhibited by inorganic materials but is a plan which, built upon the relatively simple arrangements of materials outside the protoplasmic sphere, has become amazingly elaborated and of extreme complexity.

The phenomena characteristic of life are possible only when a tremendously complex association of certain elements is present. But the materials used in the protoplasmic mélange—the atoms of carbon, hydrogen, nitrogen, oxygen, sulphur and various other elements—are the

* These four papers conclude the Symposium on the Cell Theory presented at the meeting of the American Association for the Advancement of Science at Richmond, Va., in December, 1938, the first part of which has been published in the November-December, 1939, issue of THE AMERICAN NATURALIST.

¹ Certain portions of the material in this paper have been previously presented in various addresses given at Brown University (April, 1936); Connecticut Academy of Arts and Sciences, New Haven; A.A.A.S., Denver meeting, 1937; Research Laboratory, General Electric Company; Rice Institute, and the University of Alabama.

same in living materials as elsewhere. And, as we all know, by means of the cycles of elements in nature, there is a continuous interchange of these materials from the inorganic to the organic and back to the inorganic. Now every element has its own unchanging characteristics; the structural features and functional behavior of a carbon atom or the atom of any other element is not changed when temporarily built into the protoplasm of a living organism. But the almost incomprehensible complexity of living materials, constructed from a wide assortment of elements, is accompanied by unique phenomena maintained by the utilization of radiant energy—the characteristics of the living state—which are beyond and different from those of the individual elements concerned or from any known association of elements outside the living organism. Even at present the scientist has nothing definite to offer as to when or where or how living phenomena originated, nor as to the basic features of the complex chemistry essential to life.

But turning from the functional to the organizational or structural characteristics of life a great deal of knowledge is now available, based upon contributions from physicists, chemists and biologists, which give a clear view of the elemental patterns of protoplasm: the field of molecular biology. Largely responsible for these advances are the x-ray and the ultracentrifuge of the physicist, the intensive chemical studies on proteins and other organic compounds and the cytogenetic studies of the biologist. It will be profitable to consider some of the data secured from each of these fields in an endeavor to correlate recently established facts relative to the building materials of protoplasm.

THE USE OF THE X-RAY IN THE DETERMINATION OF MOLECULAR PATTERNS

In connection with studies on the nature of x-radiation, the Swiss physicist, Laue, in 1912, suggested the irradiation of inorganic crystalline substances with x-rays in the belief that the distance between the sheets of atoms mak-

ing up the internal space lattice pattern of the crystal were of the same order of magnitude as the wave-lengths of the x-rays, which are about 1/10,000 that of light waves. If such were the case, the x-rays would be diffracted in accordance with the particular crystal pattern, just as light rays are diffracted by ruled diffraction gratings. This suggestion of Laue proved to be correct, and marked the beginning of the x-ray technique which, as developed and elaborated by the Braggs and others, soon became the most searching of all known tests for crystallinity. It may be said, in the words of Astbury, to be a "scientific substitute for human vision to compensate us for the fact that the only ether waves are some 10,000 times too coarse to show up the shape of the molecules." The use of x-rays to determine the basic molecular patterns of inorganic materials during the past quarter of a century has yielded results of the highest scientific and economic importance and, in fact, revolutionized our knowledge of the basic structure of important metals and minerals.

The structure of any crystalline substance as revealed by the x-ray is essentially uniform, that is, there is a definite characteristic unit pattern formed by the constituent atoms and molecules which is continuously repeated throughout the substance; a pattern which, though ultra-microscopic, may be determined by the proper treatment of the data obtained from the diffraction of the x-rays recorded on a photographic plate. This condition may be visualized by comparing the pattern in a crystalline substance with the visible repeat pattern in wall paper or a woven textile. Any regular arrangement of the molecules in a substance constitutes a crystal and will diffract x-rays in a definite pattern which is continuously repeated and can be identified. This repeat pattern in a crystalline substance is the "unit-cell, that is, the size of the smallest area containing the unit of atomic or molecular pattern which is repeated from end to end of the crystal."

The molecule of the chemist is usually defined "as the smallest portion of an element or compound that retains chemical identity with the substance in mass." As such,

the molecular unit of inorganic substances or even that of a much more complex organic substance is far below microscopic visibility. But x-ray studies first revealed the basic fact that the molecules do not exist as independent units in the solid or crystalline substances. The constituent atoms are all bound together to form the larger units of the crystal pattern without molecular separation. Thus to take the relatively simple example of sodium chloride: the data obtained from the x-ray plates show that in the crystal pattern present throughout this material each atom of sodium is surrounded by six atoms of chlorine, and each atom of the latter in turn is similarly surrounded by six atoms of sodium. Sodium chloride molecules, each with one atom of sodium and one of chlorine, do not exist as independent units.

The basic implications of such results from the x-ray analyses of crystals were very early recognized by Langmuir. In his now classic contribution on "The Constitution and Fundamental Properties of Solids and Liquids," published in 1916 and 1917, he stated that up until the time of the work of the Braggs on sodium chloride "it had been taken for granted that crystals were built up of molecules. But from this work . . . it is clear that in crystals of this type the identity of the molecules is wholly lost, except in so far as we may look upon the whole crystal as composing a *single molecule*."

It should be recognized that the association of the independent molecules in the development of a characteristic crystal pattern occurs only in solids. In a gas the unit molecules are entirely independent of each other except for chance contacts in their rapid movements. Molecules in a liquid are more closely associated than in a gas but even so are not bound together in a rigid crystalline pattern; they are, in general, free to move in any direction and accordingly are uniformly dispersed in a solution.

Apparently the first attempt to apply the revolutionary x-ray technique, which had proved to be so very successful in the inorganic realm, to the more complex organic compounds associated with the living organism occurred in

1921 when Polyani turned the x-ray beam upon cellulose and secured diffraction patterns which revealed the crystalline nature of this most abundant of all plant materials, and his results have been abundantly confirmed and extended. Chemists had long recognized the unit molecule of cellulose as a glucose residue, $C_6H_{10}O_5$, and also that many of these glucose residues were attached as $(C_6H_{10}O_5)_n$. The x-ray studies, by Sponsler, Clark and others, showed that the crystal pattern arose from the linkage of a large number of glucose residue molecules to form a chain. The glucose residue chains, in turn, are held together in parallel arrangement by unknown secondary valences to form a two-dimensional pattern.

The unit cell of this pattern, which is repeated uniformly throughout cellulose, measures $8.35 \text{ \AA.U.} \times 10.3 \text{ \AA.U.} \times 7.9 \text{ \AA.U.}$ and contains four glucose residues. In the micelle of cellulose, as shown by Clark, there are 6,000 glucose residues, or 1,500 unit cells, formed by an aggregation of 60 chains, each containing 100 residues. A cellulose fiber, as in cotton, consists of a great many cellulose micelles or crystals grouped spirally around a longitudinal fiber axis. In the walls of plant cells, however, it has been shown that particles of cellulose, reaching the lower limits of microscopic vision, are formed by an aggregation of 14×10^5 micelles. Seifriz, working with the Spierer lens, has observed that these rod-shaped units, or supermicellae, are arranged end to end to form long plates of cellulose.

Increasingly during the last few years x-rays and polarized light have been used to study the ultramicroscopic structure of various organic materials, particularly the proteins. It now begins to look as if the situation in the study of these protoplasmic building materials may be compared to that in the physical realm twenty-five years ago when the results from the early x-ray studies were first considered. And the results already obtained are of primary importance not only to the workers in the physical and biological sciences but to the economic world as well. Thus far the most extensive researches in the structure of the organic substances by x-rays have been made by the

textile industry in the studies of cotton, wool, silk and other fibers essential to this great industry. Of primary importance in this field are the contributions of Astbury, of the University of Leeds, England, but the data obtained by various other investigators, both in this country and abroad, have also added much that is of first importance.

Comparatively few of the almost infinite number of proteins present on every hand have as yet been subjected to the x-ray technique, but those that have been extensively studied, such as keratin of hair and fibroin of silk, show conclusively that proteins are not amorphous substances, with a "hit and miss" arrangement of the constituent materials, but that they are crystalline with a definite pattern, which, though much more complex than the carbohydrate cellulose and therefore exceedingly difficult to establish, is nevertheless decipherable by the skilled research worker in this field. Just as cellulose is formed by the linkage of glucose residues, so the proteins are constructed by a precise assemblage of amino-acid residues held together by the peptide linkage ($-\text{CO}-\text{NH}-$) to form the so-called polypeptide chains. Recent contributions by Langmuir, Wrinch and others offer evidence that the union of the amino-acid residues may not be in the form of long chains, as in the glucose residues of cellulose, but through a so-called cyclol grouping. This, however, makes no difference for our present consideration since, in either case, it is established that the proteins are crystalline substances.

It is also clearly established that not only are the non-living organic materials, such as cellulose and keratin, crystalline in nature, but protoplasm itself, present in various types of highly differentiated cells as, for example, those of muscle and nerve, has a rigid structural plan extending from molecular levels to visible cell units crystalline in nature. And even before the recent revelations of the x-rays, the geneticists, headed by Morgan, had established the definite linear pattern of the particulate hereditary units or genes in the chromosomes as a result of their controlled breeding experiments and also by the production of mutations by altering the gene pattern in the germ

cells through the use of x-rays (Muller)—a very different use of this important scientific tool than in the determination of the crystalline patterns by diffraction.

The recent studies of Painter on the giant salivary gland chromosomes and the determination thereby of their behavior in synaptic phenomena have given visual confirmation to the theories proposed on the basis of the breeding experiments. Apparently the actual stainable material of a gene is beyond the resolving power of the microscope, though some authorities are convinced that in certain instances the genes may actually be seen. At all events, it is definitely established that the genes in a chromosome are arranged in a precise pattern. Even if the x-ray had never thrown any light on protein structure, data from genetical and cytological studies would have revealed the crystalline nature of the chromosomes. Each chromosome may be regarded on this basis, as recognized by Bridges and others, as a huge protein molecule or crystal in which the genes and the accompanying substances are built into a characteristic pattern.

Thus the present situation relative to the structural pattern of the life-materials may be summarized by saying that, in essentially all instances where the new methods have been used, evidence has been obtained of a basic crystalline pattern as in inorganic solids. It therefore becomes evident once more that the difference between the inorganic and the organic lies primarily in the much greater complexity of the latter and not in a different method of construction. Many major details of the crystalline patterns of the varied lifestuffs are still undisclosed, but the foundations have been laid, and the next few years will undoubtedly record substantial progress.

The results that have been obtained showing crystallinity as a characteristic of all solids, inorganic or organic, represent another definite breach in the wall which has long separated form and structure in the biological world from inorganic organization. To the biologist who has been taught and who has then taught others that protoplasm is essentially amorphous—a sort of syrupy liquid

which flows hither and yon, or that "protoplasm is an aqueous solution in which are suspended colloidal substances of great complexity"—the results establishing the fact that the proteins, the building stuffs of life, are crystalline, just as are the inorganic solids, come as information of the highest importance, pregnant with possibilities for future major advances in biological knowledge.

MOLECULAR SIZE IN THE PROTEINS AND VIRUSES

In the previous paragraphs, attention has been given to the determination of the crystalline patterns characteristic of solids in the inorganic and organic worlds; patterns in which the individual molecules are merged. But our knowledge of the building materials of life has also been greatly increased by the study of protein molecules present in solution where the individual molecules can be studied and important characteristics determined. In this connection information relative to molecular size and orientation is of particular importance for the present discussion. The orientation of molecules may be studied in surface films formed by contact between two liquids, as seen, for example, in a film of oil on water. Molecular behavior and orientation in surface films were studied by Hardy some twenty-five years ago in connection with problems of lubrication. The experiments in later years by Harkins, Langmuir and their associates have proven very fruitful in various fields but particularly in giving accurate data of molecular size as determined by the use of monomolecular films. The very recent development of the multi-layered films of various organic compounds, including proteins, at Langmuir's laboratory has given additional results of the utmost importance to the biologist in showing just how the membranes of basic importance in biological systems are formed from repeated oriented layers of monomolecular films.

There are two other reliable sources of information, which have become available in the last few years, for the determination of molecular size. The first of these, the

ultracentrifuge, permits the determination of the size of molecules or molecular particles in solution by measuring their rate of sedimentation in response to a tremendous centrifugal force, which may be several million times the force of gravity. Finally, the invention and use of colloidal filtration membranes with a wide range of accurately graded pore sizes have yielded very important results.

The data accumulated in the last few years from these sources have made it possible to determine the molecular weights and dimensions of a wide range of proteins and viruses for comparison with cellular units. Stanley has tabulated the results of various workers and constructed an interesting and important chart to present them graphically. The data thus presented show a gradual increase in the unit size, beginning with the ultramicroscopic protein molecules and continuing with the virus and bacteriophage units to the cell of the smallest known bacterial organism, and, finally, to cellular units of microscopic visibility. Furthermore, at the two extremes of the series there is an overlapping by the virus units. Thus the smallest known virus particle, with a diameter of 10 m μ , is considerably smaller than some of the largest known protein molecules as found, for example, in hemocyanin, the molluscan respiratory pigment. At the other end of the series is the comparatively enormous unit of the Psittacosis virus with a diameter of 275 m μ , which is almost twice that of the smallest known cell, the pleuropneumonia organism, with a diameter of 150 m μ . Comparing the Psittacosis virus with larger cell types, shows it to be about one third the diameter of *Bacillus prodigiosus* (750 m μ) which, in turn, measures one tenth the diameter of the normal human red blood corpuscle (7.5 μ).

The dimensions of a considerable number of species of viruses and bacteriophages, lying between these two extremes, have been established: The data presented in the chart show that "the viruses form an unbroken series with respect to size from protein molecules to bacteria, but at either end . . . there is an overlapping. Certain viruses

are smaller than accepted protein molecules, and others are larger than accepted bacteria. It is obvious that on the basis of filterability it is impossible to draw lines that sharply divide the viruses from bacteria or from protein molecules."

Furthermore, it has been shown that the molecules of a virus protein may unite to form crystals which are visible under the microscope. Thus Stanley shows that the molecular units of tobacco mosaic virus protein are aggregated during crystallization to form definite needle-like crystals with a length of about 300 m μ . X-ray measurements of the molecular aggregates of virus proteins have been made by Bawden and Pirie (1937). They find that there "is a regular arrangement of the groups along each particle, and the sharpness of the reflections shows that these particles are sufficiently long for a large number of repeat units to be found in each. Each particle has an internal regularity of the type sometimes found in large molecules, but with the viruses it is on an unusually large scale. In this sense the particle resembles an animal or plant fiber and may be spoken of as crystalline."

Another well-known example of protein crystallization is beautifully shown in the formation of fibrin crystals in blood plasma. Observed under the dark field microscope the uncoagulated blood plasma shows an entirely dark field. Very soon, however, as the crystallization processes proceed, the molecular aggregates increase in size and attain microscopic visibility, the field under observation becoming lighter and lighter until it is soon flooded with a brilliant glow as the fully formed filamentous fibrin crystals fill the field. It has been shown that under the proper experimental conditions further fusion of the fibrin crystals will form fibrils many micra in length. Another significant and interesting example of protein crystallization was reported by Nageotte in 1927 in the case of the protein, collagen, which is the primary constituent of the fibrous connective tissues of the vertebrate body. A solution of collagen may be obtained by placing a tendon for

a time in a weak solution of acetic acid. At a pH of about 4.7 the collagen molecules in the solution of collagen will crystallize to form visible crystals of characteristic shape.

THE CELL AS A STRUCTURAL UNIT

Turning our attention to the cell of the biologist, it is important to inquire whether the newly established facts of organic structure, as just outlined, give any grounds for bringing cellular organization, which is almost universally associated with the living organism, into direct relationship with the structural patterns of the elemental materials from which it is built. In other words, is the cell considered purely as a structural unit an entirely distinct and unrelated entity, or does it represent the climax of a gradually increasing complexity in pattern possessing direct continuity with structural forms of less complexity associated with the materials of the non-living world? May it not be possible that the cell is essentially a protoplasmic crystal in which an almost infinite number of protein molecules, beginning with the genes in the chromosomes, are associated in a definite ultramicroscopic pattern characteristic of the particular type of cell? In such a condition, just as Langmuir early recognized, individual protein molecules are not present, but all are united to form the perfect unit, the complete crystalline pattern of a specific type of protoplasm, the terms molecule, crystal and cell becoming synonymous.

Possibly in a very real sense, therefore, the cell is to be regarded as a molecule of protoplasm; the least amount of this life stuff which will exhibit the characteristics of the living state, just as a single molecule of sugar or hemoglobin or virus protein is the indivisible unit of these substances. This condition becomes particularly evident in the enormous protein molecules of the viruses and the bacteriophage which have the greatest molecular weight of any known protein. Thus the homogeneous unstable nucleoprotein isolated from the phage and reported by Northrop, 1938, has a sedimentation constant corresponding to a molecular weight of about 300 millions. Stanley

has shown that the tobacco virus protein is infective in dilutions of one part to ten billions. Thus it seems probable that a single molecule of virus protein may propagate and cause infection of host tissue as would a cell of a unicellular organism.

There is a sharp divergence of opinion among the biochemists as to whether the unit particles of the viruses and the bacteriophages are merely enormous protein molecules equipped in some way for propagation by autocatalytic reactions or whether they are elementary living agents. Northrop, from his recent studies on the bacteriophage (1938) takes the position that the so-called "living reactions" of the phage units can be "more simply explained by analogy with the autocatalytic formation of pepsin and trypsin than by the far more complicated system of living organisms." He says:

It is extremely improbable that any protein can be synthesized by a purely catalytic reaction under biological conditions since it is known that under such conditions of temperature and pH, proteins hydrolyze slowly into their constituent amino acids and this process may be accelerated by a number of catalysts. If, now, another catalyst could be found which would cause the synthesis of proteins from amino acids without the expenditure of energy, a perpetual motion would result. There is good reason to believe, therefore, that the synthesis of protein requires energy and that it can only take place in a system organized to supply this energy, as in a living cell. Thus the cells of the gastric mucosa synthesize the inert protein, pepsinogen, which is transformed to the active enzyme by an autocatalytic reaction.

A similar mechanism will evidently account for the increase of bacteriophage and other viruses in the presence of living cells. The cells synthesize a "normal" inactive protein. When the active virus of bacteriophage is added, this inactive protein or "prophage" is transformed by an autocatalytic reaction into more active phage. This mechanism accounts for the fact that phage is produced rapidly only in the presence of growing cells, since only in growing cells is synthesis taking place. . . .

It appears to the writer that the assumption that the living host cells synthesize an inert "normal" protein which is changed to the active phage by an autocatalytic reaction accounts for the observed facts as well as does the far more complicated series of assumptions involved in the hypothesis that the phage itself is a living organism.

The opinion of Gortner² is quite the opposite. He states that additional data are necessary before one can eliminate finally the idea

² Reprinted by permission from "Outlines of Biochemistry," by Gortner. Published by John Wiley and Sons, Inc. Second edition, page 458.

of a living organism. If they are "autocatalytic" proteins with the peculiar property of inducing, when injected into living organisms, the production of more proteins of the same type, then they are certainly very unique materials and bridge the gap between living and non-living matter. Autocatalysis, so far as the author is aware, is usually, if not exclusively, a breaking down process, possibly a chain reaction mechanism where the energy for the reaction is passed on from molecule to molecule, once the chain has been set in motion. A building-up autocatalysis, whereby energy is stored and accumulated, is a special type of autocatalysis which involves such a radical revolution of ideas that the evidence in its favor should be very carefully scrutinized before final acceptance. The fact that virus proteins sediment with uniform velocity and that the virus activity of the preparation is unchanged after sedimentation is not necessarily evidence of homogeneity. . . .

Furthermore,

the crystallization of the virus protein may be due to a "polarity" of a living organism, since all cells and organisms have definite electrical polarities. Many colonies of the lower organisms assume characteristic shapes and forms, although the colony is composed of a great number of individual organisms. Although the weight of evidence appears to be at the present moment in favor of the virus proteins being non-living biochemical entities or systems, . . . one should not ignore the possibility that special types of living organisms may be involved in the phenomena. . . . As has already been indicated, if these units are proteins, the gap between the living and the non-living has been almost bridged.

Stanley, from his extensive studies on the viruses, has taken a middle position which, nevertheless, favors the view that they are living agents, though possibly of a retrograde or parasitic nature. Apparently the evidence at hand does not afford indisputable proof that the virus units are either living agents or non-living proteins. However, he feels that the evidence is very decisive that the functional characteristics of the tobacco mosaic are "part and parcel" of the virus protein molecule. They reproduce, they adapt themselves to certain variations in different types of living cells, exhibit "heritable" permanent changes or mutations, and they are destroyed by certain conditions injurious to living cells. Stanley has also shown that the tobacco mosaic virus fulfils Koch's postulates for a disease-producing parasite, except that the purity of the virus materials has to be established by rigid chemical methods rather than by biological tests. His view-point is summarized in the statement (1937) that:

As we go from the admittedly non-living to the admittedly living, I think there must be a transition stage where there are entities that may possess some properties that are considered characteristic of non-living things. What could fill this place more logically than the high molecular weight virus proteins that are intermediate in complexity between the protein enzymes and hormones, the wonderful properties of which we all recognize, and the system of proteins that we call protoplasm and that constitutes life. There is evidence that even within the virus group there is a gradual increase in complexity of structure from the small nucleoproteins to the more elaborate elementary-body type of virus. There is, however, no sharp break despite the fact that the structure of the latter may resemble that of a cell-type organism as much as it resembles that of the smaller viruses. I consider it unimportant whether we call the virus proteins molecules or organisms . . . I have referred to them as molecules solely because of the accident of my training as a chemist.

Possibly the condition may be described by the statement that the viruses are protein molecules, but they are of a size and inherent complexity which permit the establishment of certain functions hitherto known to exist only in living organisms at the cellular level. Just as the virus units have not attained the structural complexity present in cellular units, even so certain of the functional features associated with metabolic activities at cellular levels are largely lacking. But it is evidently of the greatest significance that the building materials of the viruses and of the bacteriophages, as shown by various investigators, are nucleoproteins which are also characteristic of the chromatin and gene complex in the cell nucleus. I recently heard a ranking biologist define a cell "as the immediate sphere of influence of a gene complex." The virus molecule may well be essentially an elementary type of gene complex with no intrinsic "sphere of influence" or cytoplasm surrounding it.

Gortner refers to the experiments of Beams and King in which *Ascaris* eggs were subjected to a force of 400,000_g and nevertheless maintained a normal oxygen consumption and development. From these experiments they concluded that either *Ascaris* protoplasm does not behave as Svedberg's protein systems or else "that spatial relationships in protoplasm are not essential to life processes."

³ Gortner, *loc. cit.*

Gortner states³ that "if no microscope had ever been developed which was powerful enough to render the *Ascaris* eggs visible, one could easily have concluded from these experiments that the *Ascaris* eggs sedimented as uniform-sized protein micelles having an enormous particle weight and that the vital activities characteristic of *Ascaris* eggs were properties of this massive protein 'molecule,' for the *Ascaris* egg would give the usual protein tests and they would appear as 'globular' proteins."

Microscope or no microscope, the possibility is apparent that the description of the *Ascaris* egg as a massive protein molecule with "an enormous particle weight" is correct; that the egg or any other cell is essentially an infinitely elaborate protein complex in which all the protein molecules are built into a characteristic crystalline pattern on the same basic plan as the amino-acid molecules are joined to form the various proteins. As Schmitt recently stated⁴ relative to the existence of a submicroscopic lattice or framework of oriented protein molecules in the cell:

This idea is an outgrowth of modern theories of protein fine structure and is a revival in a more enlightened form of the ancient idea that the ground substance may contain an organized complex of giant protein molecules and that important cell functions may be subserved by this submicroscopic cytoplasmic framework. Experimental embryologists have even suggested that . . . it may form the physical basis for the determination of polarity in cells and of axes of symmetry in the developing embryo. . . . An oriented lattice or cytoskeleton has been demonstrated in one type of cell—the neuron. Neurofibril formation results from the deposition upon this pre-existing lattice of protein material from the axis cylinder.

Thus, for example, the studies of Conklin on the protoplasm of *Crepidula*, in which he showed that the eggs consist of a fluid portion and also of a more resistant viscid portion, the basic spongioplasm, harmonizes with the more recent results.

It has, indeed, been extraordinarily difficult for the cytologist to get a clear vision of the cell as a structural unit because he has been blinded by the brilliance of its functional features and also because he has been unable to

⁴ Indianapolis meeting, 1937, American Society of Zoologists.

penetrate through the ebb and flow of the heterogeneous mixture of complex organic substances which are necessarily accumulated within the cell boundaries to keep the "wheels of life turning." As Seifriz points out, the behavior and appearance of protoplasm indicates, superficially, that it is an emulsion, but this is illusory—there is an underlying ultramicroscopic substance, protoplasm itself, now revealed by the x-ray and other experimental methods, which is continuous and highly organized. All the protoplasmic phenomena have their source in this ultramicroscopic organization from the complex chemistry of life to structural formations of incredible beauty and exactness, from the diatom shell to identical twins. Furthermore, the cell, periodically undergoing mitosis, reveals a temporary crystallinity at the levels of microscopic visibility with both nuclear and cytoplasmic elements united in the complete pattern. The protoplasm in a cell in the intermitic periods is not less organized; the materials are only woven into an ultramicroscopic pattern of different design. The proteins of the cell protoplasm, essential to the maintenance of life phenomena, form a perfect whole; there are no separate molecules; all are joined in a microscopically visible macromolecule, the cell.⁵

In the unicellular organisms a single "cell-molecule" constitutes the complete living unit. Each is as independent as the molecules of a gas floating through currents of air. Far different, however, is the condition in the multicellular organism where differentiation and cellular specialization hold sway. Just as it is impossible for the genes or the chromosomes or the nucleus or the cytoplasm to remain alive outside the cell complex, so it is impossible for the differentiated cells to maintain themselves outside the organism. The differentiated cells are no longer independent units, but they are bound together in the complete organism. Is it merely a superficial analogy to say that the "cell-molecules" associated in the multicellular organism are only repeating the condition of the protein mole-

⁵ Cf. Warren H. Lewis, *Science*, 89: 2314, 400.

cules which are bound together in the protoplasm of each cell to make the functional cellular unit?

The geologists long held to the Catastrophic Doctrine, the primary assumption of which was that "natural forces were more active and powerful in the past geological ages than they are now; that great convulsions of nature had riven the crust asunder into valleys and elevated other portions into mountains." But by the middle of the last century the Uniformitarian School gained the ascendancy, largely through the influence of Hutton and Lyell. On this view it was held that the geological processes had never differed intrinsically from those of the present day. And why should it ever have been thought otherwise, we now ask, since the same primary forces and elemental materials were at work? Is not biology at present, near the middle of the twentieth century, in much the same position as was geology a hundred years ago in failing to observe that there is a Uniformitarian Principle pervading all organization in the inorganic and in the organic as well? The basis of separation between life and non-life is dependent on the *degree of complexity* and not on a difference in *kind*, since the same materials are used in both domains, and they must conform to the same elemental patterns. From the simplest substance in the inorganic world to the most complex patterns of living substance there must be a graded series. Should the processes of evolution begin at the level of the living organism? Were not the union of hydrogen and oxygen to form water; the union of carbon and oxygen to form carbon dioxide; the union of water and carbon dioxide to form sugar; the addition of other elements to the glucose molecule to form protein; were not all these merely stages in the evolutionary processes which have led to ever-increasing complexity and have reached their climax in the world of life?

Needham, in his thought-provoking volume, "Order and Life," quotes Sapper as follows:

We now stand before a problem . . . namely, how is the origin of pattern in material objects in general and in living things in particular to be ex-

plained? Is it not indeed inconceivable that properties should be found in the material complex, which are not the result of the summation of the properties of the components? Are we really forced to the assumption of some supra-material, hyper-individual factors, in order to account for the appearance of the qualitatively new in the organized patterns? In my view there is only one way to picture the organization of the material complex without having recourse to such assumptions; and that is to assume that the qualitatively new in the pattern derives from the properties of the elements involved, but that certain of these properties can only come into operation in connection with certain stages of complexity. There is of course no proof available for demonstrating the rightness of this viewpoint. But it will not be denied that it describes the facts in the simplest way and has the advantage of agreeing with the analogy from the social life of man. . . . If one disagrees with it, one has the choice, *either* of seeking to contest the facts of the existence of additive properties in complex patterns, *or* of regarding them as fundamentally inexplicable and unintelligible.

Nevertheless, there is the possibility that a living organism is something more than the sum of its parts. Such being the case, it is not to be expected that "continual fragmentation will of itself necessarily reveal the true inner meaning of life processes." Since the establishment of the cell theory, living phenomena have been centered in the cell, but now it is apparent that the virus and bacteriophage particles, far below the realm of cellular organization, exhibit certain of the living phenomena characteristic of the cellular level. Accordingly, one may hold that in the intramolecular organization of the virus protein molecule with its "chemical combination of nucleic acid and protein of unusually high molecular weight we have sufficient organization within a single molecule to endow it with the lifelike properties that characterize it." Can it be established that the virus protein molecule is the ultimate and indivisible unit of life? Is there not the possibility that there is something deeper and more fundamental than cells, molecules or even atoms that contains the key to the mystery of life, some as yet undiscovered common factor which underlies all living phenomena and which, in its effect, might be compared, for instance, with the molecular theory which has brought the diverse phenomena associated with the solid, liquid and gaseous states of matter into a unified whole?⁶

⁶ Cf. Heyl, "The Lingering Dryad."

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THE PRESENT STATUS OF MITOSIS

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THE subject of mitosis is one which perhaps should not be mentioned too loudly in the celebration of the names of Schleiden and Schwann. Both men, and Schwann in particular, seem to have carried in their minds the idea that new cells arise by a sort of precipitation or crystallization. In Schleiden's formulation of the idea, a granule (the nucleolus) is differentiated out in the cytoplasm of an already existing cell. A membrane is here precipitated and lifted off by the intrusion of liquid between the two. This represents the nucleus around which, in turn, the cytoplasm is formed in a similar manner. New cells were thus thought to be formed on the inside of older cells. Schwann accepted this idea but suggested that cells may also be formed in the structureless substance outside of a mother cell in what can be described as nothing but a process of organic crystallization.

This conception of the reproduction of cells was recognized as false within a very few years, and by 1846 the work of the botanists Mol and Nägeli seems to have definitely established that cells arise by some kind of division of other cells. Among zoologists recognition of this fact was long delayed, and it was not until 1858 that the work of Kölliker, Remak and others culminated in the uncompromising position of Virchow that all cells arise by the division of preëxisting cells.

Almost as soon as this generalization became accepted, the mechanism of the process of reproduction became the subject of investigation and hypothesis. I shall make no attempt here to trace the discovery of facts or the development of ideas bearing on the question from those days to the present, but the alternating periods of satisfaction and disillusionment have irresistibly brought us to the realization that the process of mitosis is a very complicated one. It is therefore a distinct advantage in our attack on the

problem that for working purposes the division of cytoplasm can be held more or less independent of the division of the nuclear elements. I need not give here in detail the justification for this statement, but certainly there are cases in which the cytoplasm may divide without involving the contained nucleus or, indeed, in the entire absence of a nucleus. In still others the chromosomes may undergo mitosis without an accompanying division of the cytoplasm.

In view of the technical difficulties of analyzing the division process, it is perhaps not surprising that in hardly any other field have workers sought their solutions in analogies to such an extent as here and, it may be added, that in few fields have analogies caused so much trouble.

CYTOPLASMIC DIVISION

The survey of our knowledge concerning the division of the cytoplasm justifies an attitude of optimism. Even though most cells are wretchedly small, the egg cells of some species furnish somewhat better material, it being possible to subject them to experiment under controlled conditions. It is therefore not surprising that we have some rather well-established findings at our disposal which entitle us to prophesy a final solution in the not too distant future. Thus we know from the work of such men as Bütschli, McClendon and Spek that there are definite diffusion currents that intimately involve a redistribution of the surface layers of the egg (the hyaloplasm). Ingenious analogies have been presented which show that alterations in surface tension set up diffusion currents which may bring about division in a drop of homogeneous liquid.

Again, the work of Heilbrunn and Chambers has established that there is a definite cycle of viscosity changes in the course of a mitosis. In this connection Gray has shown that an increase in the extent of two viscous centers may bring about the division of a homogeneous drop that contains them.

Either action may be, and probably often is, linked with

the formation and action of asters and it somehow seems that a drawing together of our information should suggest at least the general outline of an answer to the question. The difficulties that are still preventing this arise from various sources. Thus, as has been stated, nearly all experimental work has been on eggs which, in some respects, offer rather specialized conditions. Again, the analogies that I have mentioned always utilize homogeneous substances such as are certainly not found in the living cell. But all in all it seems to me that we are not far away from a preliminary solution of the problem, even though many details will undoubtedly cause further difficulty.

DIVISION OF THE NUCLEUS

But, when it comes to the analysis of nuclear division or, to state it more exactly, the mitotic movement of chromosomes, no such optimism seems justified. This attitude is in striking contrast to that of the 1880's or even the early 1900's, when even a casual study of the amphiaser suggested strongly that the motion of the chromosomes to opposite poles must somehow depend on the activity of the fibers that attach to the chromosomes on the one hand and to the centriole on the other. The simplest and most obvious interpretation was that these fibers, anchored at the center, shorten or contract and thus pull the chromosome to the pole. An alternative hypothesis was that the interzonal connections expand so as to push the chromosomes away from each other toward the poles. However, doubts soon arose as closer analysis showed that the fibers could hardly be considered as simple elastic strands for, though doubtless they shorten, they never seem to thicken in the process.¹ And for the second hypothesis it was pointed out that if a pushing action is given by an expansion of the interzonals, an additional force must be involved that guides the chromosomes toward a focal point

¹ Professor Conklin informs me that he is convinced that in at least some forms such thickening nevertheless does occur.

at the pole. There were additional complications that lay in the position taken by chromosomes on the equatorial plate; the question of how fibers growing out from one center met other fibers coming from the opposite center to make continuous or primary fibers; how still other such fibers managed to make connections with the chromosomes, and so on and so on.

Things became so complicated and involved that investigators who were looking for a simple, comprehensive explanation for observed phenomena were seized with a kind of despair which in turn resulted in a tacit agreement that the matter had better be laid on the shelf until advances in other fields might suggest a new attack from another angle. It was this attitude that prepared the ground for the advent of the so-called dynamic hypothesis of the early 1900's.

The obvious resemblance of the amphiasster to an electrostatic field of force had been noted often enough, but it remained for Hartog, Gallardo and R. Lillie to construct working hypotheses on this basis. These men reasoned that if the two centers carry opposite charges the conditions that bring about the resemblance to an electrostatic field are present. But this was too good to be true, and almost at once very valid objections arose. The configuration of amphiasstral rays did not really conform to that of the lines of force postulated, for they frequently crossed and anastomosed. Again, the existence of tripolar and quadripolar figures offered the difficulty of amphiasstral figures between poles of like sign. Multiple asters always repelled each other; that is, they always seemed to carry the same charge. Hartog's and Gallardo's efforts to escape from these difficulties are marvels of ingeniousness, but the fact remains that the various objections could not be overcome without an extensive adoption of additional and subsidiary assumptions. And, when everything was said and done, such constructions were usually of little aid in regard to our present question, for they involved the chromosomes only in the rôle of minor agents, if at all.

It was left for R. Lillie to round out the picture. There is not time to elaborate on what was in the end a skilfully constructed hypothesis. In its merest outline it postulated that the centers are of like sign, as mutually repelling asters indicated, and that the chromosomes take an equatorial position because they carry a charge of the same sign as the centers and are thus repelled from the opposite points toward a mid position. They are kept there because this middle region of the spindle carries a plus charge. Then, as each chromosome divides, the daughter chromosomes repel each other and the initial anaphasic movement is thereby given. But then arose a great difficulty. In completing a movement to the poles the chromosomes would perforce be moving against the repelling force of the centers, which originally had been powerful enough to force them into an equatorial position. There was thus no escape from the conclusion that this final movement presupposed a change in charge on the part of the center which altered it from a repelling to an attracting, or to at least a neutral, point.

Now it must not be forgotten that reasoning by analogy had of necessity played a large rôle in this hypothesis, so that the cautious cytologists of the day had never felt altogether safe in following Lillie. And thus, when in addition to this Lillie postulated a shifting or alteration of electric charge, most cytologists refused to follow. The matter was finally dropped by common consent as being altogether too involved and complex.

The abandonment of the dynamic hypothesis seemed to close the last avenue toward solution and for some fifteen years (roughly from 1912 to 1927) the whole problem lay virtually untouched. In the latter year Bělař reopened the question and his starting point was the pushing force involved in an expansion of the interzonal region between separating chromosomes. He followed this beginning with a series of brilliant investigations, but after some four years he reached a position that was surprisingly old-fashioned and new only in the sense that it brought together several opposing old hypotheses. In essence it

postulated a combination of the pushing force of Drüner, as given by the expansion of interzonals (Bělař's *Stemmkörper*), and the pulling force of Van Beneden, as given by the gliding and contraction of certain other elements in a poleward direction (Bělař's "*Zugfasern*"). But what forces were involved in the expansion of the *Stemmkörper* and what in the contraction and movement of the half spindle elements were questions that Bělař left for the future. However, his work had served as the stimulus to fresh courage and to a renewed attack on the problem.

Lamb's demonstration of the action of the oscillation of two isolated centers now received reconsideration, and certain other hypotheses were advanced. Thus may be mentioned that of Schaede, which utilized the streaming of protoplasm as a moving force; the conclusions of Bleier and Metz accrediting autonomous powers to the chromosomes and involving accompanying action in the protoplasm surrounding them; Wassermann's idea of viscosity changes originating in the equatorial region and pushing daughter chromosomes toward opposite poles. Objections can be voiced to all these suggestions, and admittedly not any one of them is a finished product. But whatever their future fate may be, these various arguments and renewed investigations (and many seemingly irrelevant observations) have made us more certain of some of the dubious facts of 1905, have definitely eliminated others, and have finally brought forth some new ones.

One of these early observations now made secure is that the initial separation of daughter chromosomes is independent of the amphiaster. Whether the split chromosome is near the spindle or not, whether it shows spindle fibers or not, be it at right angles or parallel to the spindle axis, when the time for anaphasic movement arrives the daughter chromosomes separate and go in opposite directions. The process certainly is autonomous, and there is no simpler way to describe it than to say that the daughter chromosomes appear to repel each other.

Similarly, there seems no escape from the conclusion that whole chromosomes also repel each other, as evi-

denced not only by their position in the late prophasic nucleus but also by their behavior toward each other on the metaphase plate.²

At the same time the cataphoresis experiments of Lillie, Dahlgren, Pentimalli and Churney and Klein seem to demonstrate that the chromatin of the nucleus is electro-negative during most phases of the mitotic cycle. I use the word "seem" advisedly, for in such experiments the danger of alterations in the affected cells due to injury from the electric current is very difficult to control. But taken as they stand, if these findings may be drawn together then at least the initial movements of chromosomes might well be ascribed to the charge carried by them.

Again, the old observations on the centers still seem valid. When the centriole divides, the daughter centrioles separate and travel to opposite sides of the nucleus, their paths being delimited only by the seeming necessity of remaining within a certain distance of the nuclear membrane. I may say here that it seems to me a minor issue whether a centriole be visible as a morphological entity or not. Certain it is that some centralizing factor is present even where no centriole can be demonstrated.

More recently we have discovered that every chromosome exhibits a specially differentiated region whose optical properties and staining reaction suggest a relationship with the centriole. This body of many names—let us call it the kinetochore—is without question deeply involved in the mitotic movements of the chromosome. Except in some very special and rare instances it is this part that precedes in the advance of the chromosome toward the pole. However, if the relationship that is so evidently suggested by this movement be one of attraction between centriole and kinetochore it should be pointed out that it is not always present throughout a mitotic cycle. Thus there is every indication that during a certain phase

² Though perhaps far-fetched it may not be amiss to point out that a mutual repulsion of bodies is open to at least one other explanation than that offered by electrical charges. Thus two bodies may repel each other if both are diffusing strongly—as in the case of two pieces of camphor floating on water.

of meiosis, the bouquet stage, it is not the kinetochore region that shows attraction toward the centriole but the free ends of the chromosome. It is therefore patent that either the kinetochore or the centriole is subject to certain changes that affect their relationship to each other.

I am drawing attention to these observations because they once more point to a dynamical hypothesis such as was proposed by Lillie in 1905. To be sure the difficulty encountered by Lillie is still present. If it is assumed that chromosomes and centers are negatively charged, the initial separation of daughter chromosomes may well be due to mutual repulsion. But the last part of the anaphasic movement still remains to be explained, for, as already indicated, it would take place against the force exerted by the negative center. So far as I can see, there are just two ways of escape from the difficulty: one, that the negative charge of either chromosome or center changes to a positive one or else becomes weak or neutral, while the movement is going on; the other, that, unlike the main mass of the chromosome, the kinetochore is positive and thus is not repelled by the center. But analysis will show that even in the latter contingency some degree of alteration in the charge would probably be called for. In short, the key seems to lie in the possibility of a change in the electric charge of one of the elements involved while the process is going on. I am not sure that this is altogether impossible.

No matter how we may twist and turn, however, we are faced with the realization that the whole process is very complex. Darlington, who has recently attempted an explanation on these lines, is forced to make so many assumptions that I do not feel very safe in following him. It must be remembered that every dynamic hypothesis has to reckon with factors that are given by the structure of the cell and these may be independent of any electrical action. Thus the expansion of the interzonal region seems an extremely important part of mitosis in at least some forms and I can not easily use it as a cog in any dynamical hypothesis. Again, certain forms point to a multiple

origin of the spindle, which is at present difficult to reconcile with the claim that the centers carry a charge. But such difficulties are self-evident, and I mention them merely to show that an explanation resting solely on electrical phenomena is going to encounter difficulty. Indeed the necessity for much further work is self-evident, for there are few problems that so urgently call for more well-established facts.

Finally, I should like to mention valid objections to any hypothesis involving the mechanism of a polarized or electrostatic field. These objections lie chiefly in the existence of lines of force in a system with three poles. Tripolar spindles certainly exist and the diagonal spindles of quadripolar figures also have a bearing. But in this connection it may not be amiss to point out that currents or lines of force such as are implied by an electrostatic field are not absolutely necessary to the hypothesis. Teorell has indicated that in an aqueous system where an electrolyte is diffusing steadily, an electrically charged particle like a chromosome would certainly be moved. If it is negative it would progress toward the opposite part of the diffusion potential field, and *vice versa*. Now in such a system no cataphoresis effect is involved and no current is flowing. This might remove some of the basic difficulties encountered by the electrostatic set-up that I have mentioned and yet not alter the rest of the reasoning.

I should like to repeat what I have said earlier: We must reconcile ourselves to the realization that the whole process is complicated. Even if electrical forces are mainly involved, there can be no question that modifications due to cellular conditions will have to be taken into account. Perhaps I am not altogether devoid of hope because I have become resigned to the thought that a solution will not be made by a single brilliant stroke. We all hope to make such a brilliant stroke in the course of our work and it is not without a certain sadness that one sees the possibility fade. But it may be that there is here at last an instance where a philosophy of resignation may lead us out of the wilderness.

THE PROBLEM OF CELL INDIVIDUALITY IN DEVELOPMENT

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BEFORE considering its embryological implications, let us first scrutinize the concept of cell individuality in the developed organism, from which it was originally derived. When Schwann says, "Each cell is within certain limits an individual," his qualification "within certain limits" seems to disavow the doctrinary rigidity which some of his followers have injected into the cell theory when they proclaimed that anything that ever happens in an organism is the resultant of individual cell activities. The severe and scornful criticism which these extreme "elementarians" had to take from the opposing camp of extreme "wholists" who wanted the attribute of individuality strictly reserved to the organism as a whole, is too well known to need further comment here. Much of this fight was carried out on philosophical grounds rather than on the factual grounds of observation and evidence. Many arguments advanced for or against the universality of the cell concept were merely rationalizations of beliefs of their authors that an organism ought or ought not to consist of discrete elements.

It is doubtful, for instance, whether the question of contiguity *versus* continuity among the elements of the nervous system would ever have become such a perpetual issue but for the fact that one group needed membranes and the other group "through" connections to explain nervous function as they saw it. Similar preconceptions were allowed to intrude into the problem of plasmodesms, that is, protoplasmic connections between cells. Those who think of protoplasmic communication and control as transportative in nature would rather have them, while transmissive theories can very well do without them. The observed facts themselves conspired with speculations on both sides by affording the expedient of artifacts caused

by histological treatment: If a preparation shows cell bridges, one can not always be sure that they might not be coagulated intercellular matter; and if a preparation shows no cell bridges, one can never be sure that they might not have been present in the living but ruptured by violent contraction during fixation.

As these few examples indicate, there has been a definite tendency to rate cellular individuality by the criterion of morphological discreteness. Insularity of cell behavior seemed to presuppose completely closed boundaries as of an island, and the evidence for this was sought in uninterrupted microscopic delineation. Obviously such an attitude is unwarranted in view of present-day biological knowledge which has stripped the microscopically visible of its former prerogative as vital standard and has pointed us more and more toward the ultramicroscopical and molecular realm. A submicroscopical boundary along which colloidal and other physico-chemical properties of the protoplasm change is no less real and physiologically effective, even though the microscope fails to reveal its existence. Nevertheless, some of the old practices are still with us, and to this day much of the discussion of cell individuality continues to revolve around microscopical arguments.

From this angle, however, the question is unsolvable. There is as much evidence for the existence of discrete, well-demarcated cell individuals as there is for that of plasmodia, large protoplasmic continua containing numerous nuclei but no visible cell limits to fence off nuclear domains. Free migratory cells are clearly unicellular individuals; but the heart muscle is clearly a syncytium; so is the blastoderm of the insect egg—to mention only a few prototypes. The occurrence of both protoplasmic continuity and protoplasmic fragmentation has been amply demonstrated. But more than that: it has been demonstrated that either condition can change into the other. Morphological delineation has thus turned out to be a rather inconstant character and by no means a true

test of cellular individuality. Cell bridges can break and reform; symplasms can divide up into cells; and cells can merge again into syncytia. Let us choose three examples to illustrate these facts, one from the lowest and the other two from the highest group of animals.

(1) In continuation of earlier studies by Wilson, Galts-off and others, Brondsted has recently made a thorough reinvestigation of the problem of cellular individuality in the reconstitution and germination of sponges. His observations leave no doubt that cell discreteness is of a transitory character, which comes and goes according to circumstances. 'Repeatedly, cells merge into large plasmodial masses, thereby losing their outlines, and later emerge again as individualized, well-circumscribed units. Free circulation of granules and other inclusions between the formerly isolated cell body and the ground substance into which it has opened, attests to the disappearance of morphological cell boundaries in the act of fusion.

(2) The syncytial character of the mesenchyme of vertebrates has been asserted by Rohde, Hueck, Studnička and others; again it has been recorded that under certain conditions nucleated parcels of protoplasm emancipate themselves from the syncytial continuum as mobile cells which can probably later become reincorporated in the common plasmodium (v. Möllendorff).

(3) Perhaps in no other tissue has the dogma of the morphological discreteness of the cellular individual been so vigorously defended as in the nervous system. As for the embryonic origin of the nerve fibers, the neurone doctrine has come out victorious, inasmuch as the neurite of each nerve fiber has been found definitely to be the product of a single discrete nerve cell. However, evidence of secondary protoplasmic anastomoses of the individual units keeps steadily accumulating. To the best of our histological knowledge, particularly according to Boeke, the terminal branches of a motor nerve fiber pass into the protoplasm of the muscle fiber without morphological interruption. Similarly, the connection between a cutaneous

nerve fiber and its sensory end organ has been described and depicted as intraprotoplasmatic. Anastomoses among nerve fibers were observed in the living object by Speidel and in tissue culture by Guiseppe Levi, who otherwise is one of the staunchest supporters of the individuality of the neurone. In nerve regeneration the newly outgrowing nerve fibers merge with the protoplasmic syncytium of the so-called cords of Büngner and only later become set off from the matrix and from one another by insulating sheaths.

Apparently, cell contour is a much more variable character than one would have anticipated. It can fade and reappear. But does this upset the concept of cell individuality? I do not think it does. In the studies on sponges quoted above, Brondsted has reported that even after merging into plasmodia, the different types of constituent cells can still be distinguished by differences of their nuclei and perikarya, and when a cell becomes released again, it behaves true to its original kind. Evidently, cellular individuality can survive protoplasmic confluence. We may adjust our picture of the cell to this situation by de-emphasizing the criterion of delineation. The only definitely discrete element in a cell is the nucleus, and since each nucleus keeps protoplasm within a certain radius under its control, protoplasmic territories have the value of cells, no matter whether their boundaries are marked by visible surfaces or merely by a change of physiological properties along the border. Any change in the colloidal consistency of the protoplasm attended by biochemical and bioelectrical differences will necessarily produce a definite orientation of ultramicros along the boundary and create some sort of physiological barrier and some degree of physiological isolation.

Within these limits, the cell is an individual, anatomically and physiologically speaking. But what about the cell in development? Let me briefly outline the crux of this problem also.

At the end of development we are confronted with a

unitary organized system, called an "organism," which, at the same time, is a collective of cells. At the beginning of development we find just one primordial cell—the egg. We call a system "organized" when its multiple elements appear in typical diversity, typical spatial distribution and typical temporal order. The elements are subordinated to this order and their freedom is restricted by it; hence, the order is a supra-elemental property of the system. In the developed system, "organism," the cells represent the elements; hence, organization is a supra-cellular property. But the primordium of the organism—the egg—does not consist of cells. Now, there arises a dilemma. Either the egg already possesses supra-cellular organization of the same order as the later body—then it is not just another cell, but an uncellulated organism; or it is merely a cell like others—then it can not be at the same level of organization as the later body. In this case, development would create organization of a higher order. It is to this latter view that the cell theory has committed itself. In the words of Schwann, "the individual cells so operate together in a manner unknown to us as to produce a harmonious whole," and the stress lies on "produce." Cells springing forth from repeated divisions would join hands, as it were, as equal participants in the building up of an organization all of their own making. The organism would be synthesized by progressive integration of cells into higher units, tissues, organs and the body as a whole. Cells would form the organism.

This view has met with vigorous opposition, culminating in a number of pronouncements about the inadequacy of the cell theory of development. In his address at the World's Columbian Exposition, Whitman argued the case in the most trenchant manner. One must realize the philosophical implications of the problem. If organization was to be accepted as something created *de novo* in every ontogeny, some principle had to be invoked which could mold order out of chaos, and the resort to vitalistic agents, such as Semon's "Mneme" and Driesch's "Entelechy,"

was a logical outcome. Faced with the alternative, the assumption of some primordial organization inherent to the egg seemed to many a much more palatable solution. Thus, the egg was vested with organizing powers representing the supra-cellular organization of the later organism, and research was directed toward the establishment of external signs of this organization. The egg and the young germ were considered as primarily integrated wholes within which parts gradually arise by individuation. At no time would the cells constitute independent units, but from the very beginning they would be subordinated to the actions of the organism as a whole. The cells would not form the organism, but the organism would break up into cells.

Clearly the two opposing views represented a modern edition applied to organization of the old antithesis: epigenesis *versus* preformation. Epigenesis of organization was the claim of the "egg-equals-cell" theory, while preformation of organization was the tenet of the "egg-equals-organism" doctrine. The latter soon gathered momentum from experimental evidence. Lillie showed that activated eggs of a worm, *Chaetopterus*, when prevented from cleaving into cells as in ordinary segmentation, still underwent a considerable degree of differentiation, involving development of parts within the protoplasmic continuum of an undivided egg. Localized differentiations of the egg cytoplasm of ctenophores, annelids, molluscs, insects and amphibians became known which imparted definite substantial and dynamic properties upon the cells to which they happened to become apportioned during cleavage. The cellulation of the egg was gradually recognized as a sort of epiphenomenon superimposed upon the differentiation of the germ rather than instrumental in its production. More and more one became impressed by the fact that the organization of the germ as a whole has stability as such, regardless of the extensive fluctuations to which its cells are subjected in nature and experiment. The individual cells began to appear as slaves, rather than

bosses, of the organism. The existence of individual cells as units was still acknowledged, but their rôle in embryonic organization was strongly de-emphasized.

As so often happens, however, in the wake of this sound reaction to exaggerated claims of the cell doctrine, an equally intransigent anti-cell doctrine raised its head. It tried to deny cellular individuality altogether and advanced a veritably totalitarian concept of development. Cells were ignored. The mass of the developing organism was considered as clay in the hands of the sculptor, passively submitted to molding forces which neither respect internal boundaries, nor admit of constitutional autonomy of individual units. If to the extremists of one side the individual cell was all and everything, to the advocates of the other extreme the organism as a whole appeared from the beginning in unchallenged control, cells or no cells.

Experimental embryology has, on the whole, steered clear of the two extremes. But it was difficult for the issue to find its proper level so long as one put the problem in terms of an alternative: Is the egg a cell or an organism? Is development epigenetic or preformed? Do the cells establish the properties of the developing organism or does the organism determine the properties of its cells? And so on. As we now see them, these questions are about as pertinent as if one asked: Has the face of the earth developed by volcanism or by erosion? The face of the earth is a highly complex affair, and so is its development. So also is the development of an organism. The time has passed when one could speak of development as if it were a single simple unitary phenomenon, like lightning or crystallization or the casting of a mold. Experimental analysis has revealed that what, in one word, we plainly call "development," is in reality an intricate combination of innumerable component processes, diverse and often disparate in character, which merely simulate oneness in that they all affect the identical material system—germ. Of course, it has been customary all along to single out growth (meaning increase in mass) or differentiation (in-

crease in diversity) or morphogenesis (elaboration of shape) and the like; but there has been a general feeling that all these features are manifestations of a common principle, and that to separate them was pardonable only as an act of mental abstraction. The truth, however, is that they are essentially separate phenomena, and, in fact, each one in itself highly composite. Nuclear division, cell growth, cell division, cell aggregation, movements of cell complexes, differential growth, cytological differentiation, polarity, orientation—these are only a modest selection from the list of component phenomena into which we have learned to decompose development.

The revelation of the multiplicity of developmental processes and mechanisms has been a sad disappointment; for it has removed all hope of a general, comprehensive and universal formula of development. At the same time, it compels us to ask every question which formerly was aimed at development in general, separately for each one of its manifold components. We no longer ask: "Is development epigenetic or preformed?", but focus on a single contributory phase, asking: "How much of it is due to epigenetic and preformed conditions?", only to find that the answer varies with the object. It is this abandonment of the unitarian claim which has rendered us immune to both the strictly elementarian and the strictly totalitarian view, and which has steadied our picture of the relative rôle of cell and organism in development. Instead of a sweeping generalization, we expect a precise description of just how much of a given developmental phenomenon is due to active participation of the cells and how much to effects of supra-cellular order; what does a cell do, and what is being done to it, in a given phase of development? These are questions with which one can deal in matter-of-fact fashion, without even touching the sore spots of principle.

A few specific cases may serve as examples. Let us consider, first, histological differentiation. Do cells produce specific histological characters by intrinsic capacity

or through external influences? For instance, is the elaborate conducting and contractile apparatus of a cross-striated muscle fiber developed by virtue of a constitutional property of the myoblast cell or can muscular development perhaps be imposed upon any protoplasmic mass by proper influences from its surroundings, as has been claimed by Carey? Observation and experiment have answered in no uncertain terms: Cellular differentiation is founded on innate properties of the cells themselves.

To prove the point, we remove cells from the community of the organism, thus depriving them of possible outside directives. We choose cell groups of an early germ with no manifest signs of differentiation, explant them into an extraneous medium and watch their fate. Morphogenetic development remains poor. But histologically, the explanted cells differentiate with amazing perfection. As Holtfreter and others have shown, they give rise to typical nerve cells, pigment cells, muscle cells, cartilage cells, notochordal cells, goblet cells, pronephric cells, etc. These productions are absolutely definite and discrete, each cell differentiates distinctly into one type or another, and there are no intergradations, hybrids or blends between the established cell types. Obviously, even very young cells "know" how to make a muscle fiber, a neurone, a chromatophore, etc., and we may conclude that the mechanisms for histological differentiation belong to the pre-formed endowment of a cell.

The same experiments have revealed, however, that cells are by no means single-tracked from the beginning. We know approximately what is to become of any given cell group of an early germ during normal development. Now, one has often noted that cells, when reared in isolation, can deviate considerably from their normal fate. Presumptive nerve cells, for instance, can become muscle cells or chorda cells, and the like. This means, evidently, that each cell of the early germ possesses a definite repertoire consisting of several discrete differentiation po-

tencies. A limited number of clearly circumscribed courses are open to each cell.

In isolation, chance may decide which course is actually followed. But inside the organism, the choice is definite: there, each cell develops in conformity with the character of its surroundings. It becomes a cone or rod when in the retina, a cartilage cell when in the center of a limb bud, and a neurone when in the brain. Intrinsically capable of a variety of performances, the cell receives some definite cue from the locality indicating which trend it is to follow. These cues are decidedly of supracellular origin. Their effects have been beautifully demonstrated by transplantation experiments for which the schools of Spemann and Harrison have become famous. Transplant a young and undifferentiated cell group into the region of the head, and it will form eye or brain; transplant it to the anterior trunk and it will form limb, or further back, kidney; and, transplant it to the rear, and it will form tail—the same cells forming different structures depending on their locations. We may say: “Organizing factors take hold of the cells and direct them to appropriate formations.”

We must be careful not to lapse again into the erroneous metaphor of the sculptor molding clay; let us stress, therefore, that no organizing factor has yet been observed that would have made cells assume histological structures strange to their inherited repertoire. This statement is based on crucial evidence obtained from transplantations between different species and orders of animals. In provoking specific histological characters, organizing influences are bound, therefore, to operate through the cells as their executives, and the specific character of the execution is determined by the properties of the reacting cells. To this extent, differentiation is active cell work. But this is not the whole story: The factors which turn a given cell into a definite histological trend do not, at the same time, fix all the particulars of its future course. Take a nerve fiber, for example. The factor which turns an indifferent epithelial cell into a nerve cell does not, at the same

time, decide the spot at which the nerve fiber will leave the cell body; and the factor which opens the door for the fiber has no control over the further journey of the outgrowing sprout; and, again, the factors which map this course are different from those which decide where it will terminate. To put it drastically, the nerve fiber is elaborated in assembly plant fashion. In some phases of this sequence the individual nerve fiber plays an active role; in others it behaves purely passively. The original outgrowth of the sprout is free, guided presumably by oriented traffic routes of the surrounding body. But once the free tip has become hitched to a peripheral migratory cell, it is taken in tow and dragged to a destination no longer of its own choosing. It is not at all easy to tell how much of the winding course of a nerve fiber is due to active orientation and how much to passive distortion.

The same holds for cell shape in general. Part of it can be ascribed to autonomous transformations of the individual cell body, the rest to passive deformation caused by pressure, growth and spatial limitation on the part of the cell collective. With cell movements it is the same story: Free cells may aggregate in response to a local stimulus and thus form a crowd whose further growth and movement, as a whole, sweep the participant elements along without leaving them much further individual choice. Similarly, erstwhile free cells which secrete a cementing substance thereby imprison themselves and become subjected to all the dislocations of their common matrix. An opening or canal may be formed either by the active recession of the cells lining the prospective lumen or by the passive destruction of cells with subsequent resorption. When one sees pigment cells arranged in regular geometric tracts, one suspects that they have been forced into this alignment by the topography of their surroundings. But how much the pattern is really of their own making has been shown by Twitty when he interchanged the source of the pigment-forming cells between two species of distinctly different color patterns: the transplanted cells

assumed the distribution characteristic of the species of their origin rather than that of the host body. I would venture to say that what the individual cells actually bring into the deal in this instance is a tendency either toward dispersion or toward aggregation; whereas the loci of aggregation in the latter case are presumably a matter not of the cells themselves, but of their matrix, so that the resulting pattern would, again, be of composite origin.

I have deliberately dwelt on these varied examples, in order to make clear that practically every step in development reveals the cell in a double light: partly as an active worker and partly as a passive subordinate to powers which lie entirely outside of its own competence and control, *i.e.*, supra-cellular powers. Now, it is perfectly true that some of these latter result from interactions of cell individuals and are, therefore, of cellular origin. But it is equally true—and the findings of experimental embryology are one rich store of evidence for our assertion—that many of them are supra-cellular from the beginning. They are those organizing conditions through which the fate of the individual cells—undecided, as we have seen, at first—is guided, controlled and progressively fixed. They are definite at a time when the individual cell fate is still indefinite. They impose order upon what otherwise would be an anarchic cell chaos. They are inherent properties of the living system, germ, as a whole, in contradistinction to the inherent properties of its constituent cells of which we have spoken before.

One frequently refers to these organizing entities under the term of "fields." Their existence can be traced back to the egg. In fact, just as there is a continuity of cells from the egg to the organism through successive cell divisions, so there is continuity between the primordial organizing fields present in the undivided egg and the localized fields of the later germ. Primordial fields segregate progressively into more restricted fields, and, furthermore, induce new fields in neighboring areas of the germ. Thus,

the organizing principles of a germ have an ontogenetic history of their own which is not cell history. Their possession marks the egg as an entity of the rank of the organism; this, in answer to a question put above. Their development is a matter of the developing system as a continuum, like tensions, currents, potentials, and the like, and they pay no heed to cell boundaries, although sooner or later the intricate interplay between them and the cells sets in, of which we have spoken before.

The existence of these primordial organizing principles in the egg has been firmly established by modern experimental embryology. No pure cell theory derived from the developed organism can embrace them, unless by a vicious circle.

In conclusion, we may say that the cell theory is correct: The egg is a cell and it gives rise to all the successive cell generations which contribute to the organism. But the organismic theory is likewise correct: The egg is also an organism, and it passes its organization on continuously to the germ and the body into which it gradually transforms. Only this dual concept seems to fit the facts, as we see them at present. To be consistent, we should supplement Virchow's well-known tenet of the cell theory: "*Omnis cellula e cellula*," by its counterpart: "*Omnis organisatio ex organisatione*." If the former denies spontaneous generation of living matter, the latter denies spontaneous generation of organization. In admitting this, we merely paraphrase what Whitman has called the "continuity of organization." But within these specified limits the cell, even in development, is still, as Schwann has said, an individual.

WHAT OF THE FUTURE?

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SPEAKING as a philosopher, and in search of the qualities which distinguish living things from the non-living, Bergson points out that the incorporation of time into the living system is its distinguishing characteristic. He expresses the thought in these words:

Like the universe as a whole, like each conscious being taken separately, the organism that lives is a thing that endures. Its past, in its entirety, is prolonged into its present, and abides there, actual and acting.

Wherever anything lives, there is, open somewhere, a register in which time is being inscribed.

If time is thus inscribed, where is the record and how is it preserved? That question has been largely answered by studies on the structure and behavior of cells. Obviously, organisms are brief and fleeting in existence—they can not represent a continuous record. But within them are the germ cells which do constitute an unbroken and constant series. Since, however, male and female germ cells differ widely in many respects, there must be comparable structures in the two which would represent this temporal record. It was not long until such a situation was demonstrated in the nuclear structures, now for so many years the objects of intensive study—the chromosomes. They were observed to reproduce themselves down to their ultimate visible parts with the utmost accuracy. The results of this appeared in the constancy of number, size, structure and behavior of chromosomes, even in large taxonomic groups. Here there is certainly some sort of a record persisting, obviously, over a period of time estimated at millions of years. But is it the precise inscription which will delineate the characters which we associate with organisms of specific types? Genetics has convincingly demonstrated that the record is complete, specific, accurate and persistent. In the final analysis, however, there are lacking the visible ultimate units, within the chromosomes, which genetical analysis calls for.

Confronted here with the same difficulty which physical scientists meet in their analyses, the biologists solve it in the same manner by the creation of supposititious units which they call genes.

A survey of the history of the cell doctrine shows accordingly that it demonstrated: (1) The universal presence of cells in organisms; (2) the differentiation of these into two types—somatic and germ; (3) a method of exact reproduction; (4) the presence within the cell nucleus of a continuous racial material; (5) an exact parallelism between the behavior of this material in the germ cells and that of somatic characters genetically; (6) the precise organization of the racial material in a linear order within the chromosomes; and (7) an exact relation between this chromosome organization and groups of somatic characters.

A continuation of these most fruitful coordinated lines of study will undoubtedly bring notable additions to our knowledge of cellular phenomena. At the same time the extension of the analysis of vital processes to invisible causal units introduces necessarily new methods of study. Here we find ourselves on somewhat the same ground as that occupied by physical scientists. Like them we must reach some conception of the nature of these imagined units. First we have to determine whether our search lies directly with the physical scientists or whether, accepting all the aid they can give us, we finally depart into fields of our own where new guides and methods are required. Their own experience seems to point the way.

The physical scientists have discovered a series of at least three systems of increasing complexity, each having characteristic units whose properties explain the operation of the system. The units of the most complex order are constituted of those of the next, and these, in turn, of the simplest. Molecules, atoms and corpuscles are thus related. Three important things are here to be noted: (1) The genetical continuity of the series. (2) The specific relation existing between the character of a unit and

the phenomena of its system. Chemical activities are explained directly in atomic terms, but atoms can not here be dissociated from corpuscles on one hand or from molecules on the other. At each level in the organization of matter there are apparent characteristic phenomena, and to explain them there are conceived appropriate units. (3) The state of matter, or the nature of the successive combinations of units to form more complex ones, depends upon the conditions associated with the system, notably temperature. On the earth, where a relatively limited temperature obtains, atomic and molecular organization prevails.

When we come to living systems, such as cells, we encounter new phenomena, slightly or not at all represented at lower levels. To explain these, two antagonistic theories have been held. On the one hand it is assumed that vital phenomena are so peculiar that entirely new forces must be conceived to explain them; on the other hand it is postulated that they are not unique, but that the laws of physics and chemistry alone are entirely sufficient to account for them. Since living things have in them only such chemical elements as are found in the non-living, it is impossible to conceive of them as entirely unique and apart from the rest of nature. Likewise, because they manifest phenomena all their own, it is hardly possible that they should have the same organization as the non-living. If unique phenomena require novelty of organization and if the nature of an organization is dependent upon its constituent units, then it must be concluded that there are characteristic living units. Further, if these are not merely molecular, as the argument indicates, then they must be produced by an appropriate combination of molecules. Further, vital phenomena are possible only under limited and unusual conditions, realized possibly on this one planet alone.

Physical scientists in search of an understanding of their units note the constancy and limitations of the phenomena to be explained. Everywhere in the universe

appears the same limited series of chemical elements with the same precise seriation. The symbol of constancy in relations, a circular orbit, typifies the path of one unit or component about a fixed center or another sort; additions of components, increasing the complexity and weight of the whole, are sufficient to provide the series of chemical elements. Combinations of these relatively simple atoms in accord with their inner nature suffices to produce the much greater range of molecules which characterize compounds.

When the biologist turns to a survey of the properties of the system with which he works, he discovers very different conditions. Instead of constancy there is almost limitless variability in the aggregates which he studies. Where the chemist views comprehensible limitations to his elements and compounds, the biologist is confronted by a procession of almost infinite steps of gradation from simple to complex. In the inorganic world there are but slight possibilities of succession in time; in living things it is their very substance. Non-living things are relatively independent of their surroundings; living things depend for their very existence upon close and constant interaction with their environment. Finally, living systems have unique properties not elsewhere represented—metabolism, reproduction, coordination and consciousness. If, as assumed, units are so constituted as by their nature and action to explain the phenomena of systems, is it possible that atoms and molecules, as such, can account for vital phenomena? It seems impossible that this can be true. Bergson clearly states this contrary position in the following words:

"We do not question the fundamental identity of inert matter and organized matter. The only question is whether the natural systems which we call living beings must be assimilated to the artificial systems that science cuts out within inert matter, or whether they must not rather be compared to the natural system which is the whole of the universe. . . . The real whole might well be, we conceive, an indivisible continuity. The systems we cut out within it would, properly speaking, not then be parts at all; they would be partial views of the whole.

So of life and of the physico-chemical phenomena to which you endeavor to reduce it. Analysis will undoubtedly resolve the process of organic crea-

tion into an ever-growing number of physico-chemical phenomena, and chemists and physicists will have to do, of course, with nothing but these. But it does not follow that chemistry and physics will ever give us the key to life.

How can the origin of living units be conceived? There is but little positive knowledge to which we can turn for an answer to this question, but there are some suggestive facts which may point a way toward better knowledge. Since to preserve their existence such units must constantly react with their surroundings, it is not unreasonable to suppose that they owe their beginnings to such a circumstance. But if they did not exist, how could they react? Their very nature is one of becoming something different. They are made up of a few chemical elements and they depend for their existence and, therefore, presumably for their origin, upon a few definite conditions of radiation, temperature, moisture and chemical compounds. Under the impulse of these peculiar conditions it may be supposed that approaches to a specifically reacting unit were made and are still being made. Change is the essence of a reaction system and must have been determinative in its origins.

A single unit, conceivably, would be sufficient to establish a living system, for it would necessarily have the properties of the whole—assimilation, reproduction and coordination. The change in character is here always unidirectional—from simple to complex—the addition of more parts with increased diversity of reactions. When genes are studied they show a linear organization into aggregates. If the beginning of a system was a single gene we may assume that by transverse division it produced two, one proximal, the other distal, producing thus a polarity in relation to the environment. A continuation of this process, which obviously was infrequent and probably reactive, would result in a chain of genes. Such gene strings are identified with the linearly organized chromosomes. Inherent in any such an arrangement is succession or time. Some genes are younger than others, and it

may be supposed that their specific operation in the system would correspond in time.

Gene strings, as we know them, are parts of cells, and it is a far cry from a few genes to a complete cell. Far from being a simple thing a cell is very highly organized. It must have required a large proportion of organic history to evolve such a structure. Ordinarily when we seek a guide to succession in organic changes we try to find it in the sequence manifest in a phylogenetic series. Unfortunately, there is little to indicate the steps in cell development. Probably there are more than we know about, but almost certainly they will be found among low plant forms. It would not be difficult to conceive the formation of a nucleus from a chromosome, for this can be seen in many mitoses, but the origin of a cytosome does not seem to be so readily suggested.

When a system is inaugurated and operative it is not so difficult to understand its further development and increased perfection, but it is quite otherwise when the origin of the system is involved. A cell in general is a comprehensible structure. Its parts are differentiated and their peculiar functions are somewhat indicated by their relative positions and constitution. But how did they come into being and what is the nature of their integration? Why a nucleus and a cytosome? The relation between chromosomes (or genes) and body characters can be analyzed and predictions made regarding new manifestations, but how is the course of events between the single-celled zygote and the innumerable-celled adult determined?

The chief fault in our thinking with regard to the nature and function of determinative units in development comes from an over-simplification of the problem. From the beginning there has been a strong tendency to conceive a determiner as some factor of single and unique power operating only at some particular time. Weissmann carried this to its logical conclusion and imagined his determiners sorted out during development until finally only one kind remained in the completely differentiated cell.

As we now clearly perceive, the actual condition is just the opposite of this. There is no process of sorting out; characters do not result from the operation of single controls, but depend upon the effects of all of them; one determiner has many effects; these effects differ according to the time in the series at which factors operate; each change in the system is directly dependent upon those which have preceded it; time is thus an integral element of the system.

Our studies have led us from an understanding of the general nature of cells to an analysis of the structure and function of their parts. The progress so far has been steady and consistent, and it does not seem probable that any extensive revisions of factual statements will be called for. Theoretical interpretations likewise, in general, seem soundly based. What the future calls for is mainly an extension of the lines of investigation so far pursued, with much more emphasis upon a synthetic treatment of accumulated facts. In particular the coordinated activities of cell parts must be systematically studied so that a comprehension of the functioning whole can be reached. The method required here is obviously not that of avoidance, through appeals to an incomprehensible organismal concept, but a refinement and extension of the analytical treatment which has led us to our present position.

From the fact that analysis has finally brought us to the point where we can no longer see the units which seem required to explain cell functions, we are faced with the necessity of cultivating more strongly our powers of interpretation. Above all, we must comprehend the unique nature of these determinative units. If non-living units are relatively fixed, limited and precise, how are living units which present opposite characteristics to be conceived? What, in their nature, would lead to constant variability which, in general, is unidirectional in character? How are they organized to preserve a record of their experiences with elements external to themselves? Answers to such questions as these, I believe, will indicate the direction in which the cell doctrine will be extended in the near future.

THE SIGNIFICANCE OF POLYPLOIDY IN PLANT EVOLUTION¹

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OF the numerous processes which have been active in plant evolution, only one is well enough understood from the cytogenetic point of view so that we can give a safe estimate of its rôle in species formation. That is the process of chromosome doubling or polyploidy. The effects of this process have been so far-reaching, and it is so wide-spread, that any one who would have a real understanding of species interrelationships and species evolution in the majority of plants must realize the significance and implications of polyploidy and the processes that accompany it.

To understand this phenomenon, we must first of all know what are the effects of chromosome doubling without any other change in the genetic make-up of the plant. We can do this by comparing the morphological and physiological characteristics of a diploid plant with those of a polyploid that has been produced from it artificially, by doubling the somatic chromosome complement. Unfortunately, our understanding of these artificially induced polyploids is one of the weaker links in our chain of evidence concerning the nature of polyploidy. This link, however, is now being very rapidly strengthened, thanks to the efforts of Blakeslee (1937), Nebel and Ruttle (1938), Greenleaf (1939) and others, who have given us easy ways of producing polyploids by means of colchicine and indole acetic acid. The most important facts obtained from a study of artificially induced polyploids may be summarized as follows:

1. The size of the cells is increased.
2. The water content of the cells is increased, as was

¹ Paper read at a symposium before the Western Society of Naturalists at the California Institute of Technology, Pasadena, Calif., December 29, 1938.

shown first by Wettstein and his students (Becker, 1937) in mosses, Schlösser (1936) in a wild strain of tomato, and by Hesse (1938) in *Petunia*. This higher water content is associated with a lower osmotic value of the cell sap, and makes the tetraploid less resistant to frost than the diploid.

3. The evidence on growth rates is conflicting. Many workers have reported slower growth in tetraploids, but the only two quantitative experiments that have been performed, those of Fabergé (1936) on the tomato and Hesse (1938) on *Petunia*, failed to show significant differences.

4. The size of some of the organs is usually increased. This increase is most evident when diploids are compared with tetraploids. Higher polyploids do not usually show any further increase in size and may even be dwarfed.

5. The organs become shorter and broader. This is true of the leaves of *Solanum*, *Lycopersicum*, *Datura*, *Nicotiana* and other plants, and of the fruits of *Datura* and the Cucurbitaceae.

6. Hesse (1938) found that in *Petunia* the autotetraploid was less branched than the diploid.

7. The time of blooming is delayed (Hesse, 1938) and the period of blooming appears to be prolonged.

This information on the immediate effects of chromosome doubling is as yet based on relatively few plants, most of which belong to a single plant family—the Solanaceae.

In addition to these morphological and physiological effects, we know of two other very important results of polyploidy. In the case of recessive or imperfectly dominant mutations, the visible mutation rate in polyploids is very much reduced, since, as Haldane (1930) has shown statistically, the chances of getting a tetraploid homozygous for a set of four allelomorphs are much less than for the corresponding pairs in a diploid, for a hexaploid they are still less and for an octoploid practically negligible. Stadler (1929) has shown that the production of

visible mutations by means of x-rays is greatest in diploid wheats, less in tetraploid forms and least in the hexaploid.

Finally, the fertility of a plant is enormously affected by polyploidy. If the original plant is a fertile species, the polyploid derivative will be partially sterile, due to the formation of multivalent associations of chromosomes and their occasional irregular segregation. If, on the other hand, the diploid plant is a sterile hybrid, the polyploid produced from it is generally fully fertile. This last fact is the basis of the principle of allopolyploidy or amphidiploidy, the importance of which in the development of plant species can not be stressed too strongly. Equally important is the well-known fact that tetraploids are not only difficult or impossible to cross with their diploid progenitors, but also produce practically sterile triploid F_1 hybrids when crossed with them.

This brings us to the all-important fact, now so well known that I need not cite the numerous examples of it, that by means of chromosome doubling or polyploidy a sterile hybrid can produce directly a fertile, constant species. On this fact is based the chief classification of polyploids into autopolyploid and allopolyploid types. The definition of these terms, and the identification of the types which they represent has been subject to widely differing opinions among cytogeneticists. I believe that for practical purposes the best definition of an autopolyploid is a polyploid of which the corresponding diploid is a fertile species, while an allopolyploid is a polyploid containing the doubled genome of a more or less sterile hybrid. These are essentially the same definitions as those given by Lilienfeld (1936), but differ from those of Müntzing (1936, p. 311) and Darlington (1937, p. 183) in that less emphasis is placed on structural differences between and the types of pairing of the chromosomes. The criterion most often used for distinguishing between auto- and allopolyploids has been whether or not the polyploid forms multivalent associations of chromosomes at meiosis. This can, however, be very misleading, since we now know many more or less

sterile diploid interspecific hybrids in which the chromosomes pair almost or quite normally (*Primula kewensis*, *Tragopogon pratensis* \times *porrifolius*, *Paeonia albiflora* \times *tenuifolia*, *Crepis rubra* \times *foetida*, *Tradescantia*). These might be expected to produce allopolyploid derivatives which would form some multivalents. One of these allopolyploids, *Crepis rubra* \times *foetida*, may actually form a complete set of five quadrivalents (Poole, 1931). On the other hand, some autopolyploids, such as the tomato, may form very few quadrivalents (Humphrey, 1934; Upcott, 1935).

The difficulty with the definitions given above is that they are very difficult to apply. We can safely call a plant an autopolyploid if we know its origin from a fertile species, and are probably justified in calling it one if it falls within the range of variation of a diploid population that is interfertile or if it differs from such a population only in those characteristics known to be affected directly by chromosome doubling. An allopolyploid, likewise, can be identified if its parentage is known or if the identity of the parent species can be inferred on morphological grounds. Between these recognizable types, however, there is a long series of intermediate polyploid types, while still other polyploids are of completely unknown origin. Many of these can not be safely classified as either auto- or allopolyploid. In spite of this difficulty of definition, however, we must always keep in mind the fact that some polyploids are of hybrid origin, while others, often closely related to them, are not.

Bearing in mind these facts, we can now consider the effect of polyploidy and hybridization on a group of species. The simplest case, that of a single species and its autopolyploid derivative, is illustrated by the watercress, *Nasturtium officinale* (Manton, 1935). In this case the polyploid is morphologically within the range of variation of the diploid, except for some size differences. There are physiological differences, such as the slower rate of development and later time of blooming which have been found

in some artificially produced autopolyploids, and these give the polyploid race a different geographical range from that of the diploid. The two races have always been placed within the same species by systematists, and the question of whether they are to be considered as belonging to the same or to different species depends simply on how we define our species concept. The tetraploid is, of course, genetically isolated from its parent diploid, and might therefore be expected to pursue an independent course of evolution. This course is, however, unlikely. As I have mentioned, there is both theoretical and experimental evidence to indicate that mutation, as well as any other known evolutionary process, such as structural changes in the chromosomes, produces its effect on autopolyploids very slowly as compared to diploids. Furthermore, due to the fact that polyploid species are more infertile than their diploid prototypes, an autopolyploid is unlikely to maintain its purity unless it is completely isolated not only from its diploid progenitor, but from its polyploid relatives as well. Would such a completely isolated race, which would have to become highly inbred, and in which the visible mutation rate is greatly reduced, be likely to give rise to a new line of evolution? I doubt it.

The next case is that of a simple allopolyploid between widely different species, neither of which has in nature given rise to autopolyploid races. Examples are the classical cases of *Primula kewensis*, *Galeopsis Tetrahit* and *Nicotiana Tabacum*. In these cases, a new species has arisen at once. This species is completely distinct from either of its progenitors, and though it for the most part combines the characteristics of the latter, the new allopolyploid may occasionally possess some distinctive characteristics of its own. Since such allopolyploids contain fewer duplicated genes than autopolyploids, their visible mutation rate should be more rapid, although Stadler's (1929) experiments on *Triticum* indicate that it is nevertheless slower than that of diploids. Since, furthermore, allopolyploids usually have different geographical

ranges from those of their diploid parents, they are subjected to a different selective activity of the environment, and might therefore be expected to give rise to new morphological types through the processes of mutation and natural selection. The frequency with which this has taken place can be estimated from the proportion of genera and larger plant groups for which an allopolyploid origin can be inferred, either because their basic chromosome numbers are multiples or sums of lower numbers existing in related groups or because their basic numbers are so high that polyploidy is the best a priori explanation for their origin. The number of these genera is not large; they form about 16 per cent. of the genera of Angiosperm which are well enough known cytologically so that any inferences can be made concerning them. Most of these genera, moreover, are in complex families like the Rosaceae and the Malvaceae, and have close relatives with lower chromosome numbers. Probably the largest and most diverse single group of plants of which the allopolyploid origin is clearly established is the subfamily Pomoideae of the Rosaceae, containing the apples, pears, loquats, *Sorbus*, *Crataegus*, *Cotoneaster* and several other familiar genera. The basic haploid number nineteen, found in the poplars, willows, magnolias and grapes, the number twenty-three found in *Fraxinus*, the ash, and forty, the basic haploid number of *Tilia*, the basswood, are very likely of polyploid origin, but such cases are not the rule in the higher plants. Allopolyploidy appears to have given rise sporadically to new genera and perhaps even families, but does not seem to have been a significant factor in the production of the major plant groups.

The most complex, but probably also the most common situation in plants is that in which both auto- and allopolyploid types exist together in the same complex of related species. In this case hybridization between auto- and allopolyploid forms is almost certain to take place, and many of these secondary hybrid derivatives will be fertile enough to be self-perpetuating. If three, four or more

diploid species are able to form allopolyploids with each other, as is generally the case, additional allopolyploid types containing genes from three, four or more different diploids will be formed. Thus there arises a complex network of interrelated forms, which defies classification according to the usual concepts of the species. This has been designated by Professor Babcock and myself (1938) a polyploid complex. Such a complex consists of two or more diploids, which are well isolated from each other genetically, which therefore are, when taken by themselves, quite distinct from each other. Among the polyploids there may be autopolyploids which are nearly or quite impossible to distinguish from one or other of the diploids, except by counting their chromosomes; there are allopolyploids that are exactly intermediate between two diploids, and there are all sorts of secondarily derived polyploids. Thus among the polyploids the gaps between species no longer exist or are at least very much smaller and harder to recognize. Polyploidy, therefore, tends to break down genetic barriers and to permit exchanges of genes between genetic systems that in the diploid condition are completely isolated from each other.

As an example of a polyploid complex we may use the American species of the genus *Crepis*. In this group the complexity is increased by the presence of apomixis. This causes the polyploid portion of the complex to be split up into innumerable microspecies and permits the preservation of many "unbalanced" polyploid types—triploids and pentaploids—that in a sexually reproducing complex would be sterile and unable to perpetuate themselves. Otherwise, the interrelationships between the various types in an agamic polyploid complex, like that of *Crepis*, are essentially the same as those in a sexual polyploid complex. The *Crepis* complex has been described in detail elsewhere (Babcock and Stebbins, 1938). Other polyploid complexes are the genera *Zauschneria*, *Rosa*, *Rubus*, many sections of *Potentilla*, *Antennaria*, *Taraxacum* and scores of others. All of them are "critical" genera to the systematist. The

difficulty of these genera is intrinsic. The systematist need have no inferiority complex about his failure to find clear differences between species in them. Their complexity is the result of the preservation of scores or hundreds of allopolyploid and part allopolyploid types which are of hybrid origin, and which completely obliterate the gaps between what were once distinct or even remotely related species.

When we turn to the geographic distribution of a polyploid complex, we find this striking fact. In every case that has been studied, the majority of the diploid species are relatively restricted in distribution, while most of the widespread types are polyploid. In *Crepis* only one of the seven diploids has a wide range; the rest are confined to two small areas near the western edge of the range of the group as a whole. Furthermore, the strictly autopolyploid forms are almost as restricted in distribution as the diploids, while the farther edges of the range of the group are occupied by allopolyploid forms of complex origin. There is considerable evidence to indicate that these allopolyploids have been able to occupy a wide range because they have acquired a favorable new combination of the different physiological characteristics of their diploid ancestors. A good case of this is the *Crepis* population of the Mt. Hamilton range, in west central California. The climate of this part of the coast ranges differs from that of any of the regions occupied by diploid species of *Crepis* by possessing the combination of a mild winter and a hot dry summer. The two *Crepis* races found there are both allopolyploids, which, judging from morphological comparison, are derived from hybrids between one diploid that prefers a mild but relatively moist climate and one that grows in a cold, dry one. The Mt. Hamilton allopolyploids appear to have acquired tolerance of a mild winter from one parent and of dryness from the other. In other words, natural hybridization and natural selection have produced new types which grow under different surroundings from the old ones, just as the plant breeder produces new, better growing types by means of hybridization and artificial selection.

✓ The relative importance of chromosome doubling alone as compared with recombination through hybridization as a means of increasing the range of the polyploid complex probably varies in different genera. In *Crepis* allopolyploidy is the important factor, while in *Tradescantia* (Anderson and Sax, 1936) the increase in distribution is the result primarily of chromosome doubling or autopolyploidy.

✓ The center of distribution of the diploid species of a polyploid complex is naturally the center of variation of the complex as a whole. In the regions where two or more diploids grow together, new elements may constantly be added to the complex. In the polyploid complex of *Crepis*, for instance, the species are particularly variable and difficult to classify in northern California and adjacent Oregon; farther east and south the variants become fewer and fewer. The position of this center with reference to the complex as a whole varies in different genera; the relative distribution of diploids and polyploids is not always the result of the same climatic and ecological agents. In ✓ *Tradescantia* the diploids are chiefly southern and the polyploids northern, while in *Vaccinium uliginosum* (Hagerup, 1933) and *Campanula rotundifolia* (Böcher, 1936) the diploids are arctic and the polyploids more southern. In the Japanese species of *Chrysanthemum* (Shimotomai, 1933) the polyploids are maritime and the diploids inland types, while in the English forms of *Erodium cicutarium* (Warburg, 1938) the reverse is the case. In *Crepis*, the different diploids occupy widely different extremes of ecological habitats, and their center of distribution in northern California is a region in which several such habitats are found side by side. One important generalization which undoubtedly holds good for the bulk of polyploid complexes, including *Crepis* and *Tradescantia*, is that the polyploids are dominant in regions which have been only ✓ recently opened to occupation by plants, or which have been subjected to great climatic or other environmental changes, while the diploids tend to occupy the older, more

stable habitats. This makes the study of polyploid complexes very important from the standpoint of plant geography.

The distribution of polyploidy and polyploid complexes through the plant kingdom is strikingly irregular, and, except for its great frequency in certain families, shows no correlation with any phylogenetic scheme (Stebbins, 1938). Polyploidy is particularly frequent in the Gramineae, Polygonaceae, Nymphaeaceae, Rosaceae and Malvaceae, and in certain genera or tribes of the Ranunculaceae, Cruciferae, Labiateae and several other families. On the other hand, there are a few families, such as the Fagaceae, and several large genera (*Ribes*, *Ficus*, *Berberis*, *Philadelphus*, *Medicago*, *Lathyrus*) in which polyploid series are rare or unknown. In another group of families of monocotyledons, chiefly the Cyperaceae, Juncaceae and Iridaceae, the presence of polyploidy is obscured by the large number of aneuploid or non-multiple series found in them. On the other hand, there is a definite correlation between polyploidy and the habit of the plant (Stebbins, 1938²). Polyploidy is most frequent in herbaceous perennials and relatively infrequent in woody plants and in annuals.

Finally, we must ask what is the evolutionary history of polyploid complexes and what relation does this bear to the broader aspects of the evolution of genera and families of plants? In *Crepis* the answer is quite clear. Although there is much evidence to show that this complex has existed since the late Tertiary epoch, its polyploid members have not in all this time evolved any new characteristics. Although each diploid has some characteristics not pos-

² In this article the values for χ^2 were unfortunately calculated by the wrong method. The correct values obtained by the method recommended by Fisher, which included the combining of classes as given in Table I where these were less than 10, are as follows: Page 192, lines 44-46: $\chi^2 = 1.98$, $n = 2$, $P = 0.3-0.5$. Lines 49-50: $\chi^2 = 4.61$, $P = 0.05-0.1$. Last two lines of P. 192: $\chi^2 = 12.21$ and 13.54 , $n = 2$ (columns for 0-25 per cent. and 25-50 per cent. combined, also those for 50-75 per cent. and 75 per cent. +), $P = 0.01$. P. 193, line 5, $\chi^2 = 8.1$, $n = 1$ (columns combined as in last), $P = 0.01$. Lines 7-8: $\chi^2 = 4.2$, $n = 1$, $P = 0.02-0.05$.

sessed by any others of the diploids in this group, the characteristics of the polyploids can all be explained on the basis of chromosome doubling and of recombination of the characteristics of different diploids. *Crepis*, however, is a poor example from which to generalize, since the presence of apomixis would in itself tend to produce stability. More sexual polyploid complexes need to be studied from this point of view before we can tell whether *Crepis* represents the usual range of variability among the polyploid members of a complex. The sexual polyploid complex of *Paeonia* seems, according to my present knowledge (Stebbins, 1939), to hold to the same rule, in spite of the fact that it is obviously very old. In any case, however, the odds are against the polyploid complex as being the originator of any really new line of evolution. As compared with a group of diploid species, a polyploid complex tends to be a closed system. It can produce endless new species, but these are all or nearly all new combinations of the same supply of genic material; they are new variations on an old theme. There are hundreds of species of *Rosa*, *Rubus*, *Potentilla*, *Senecio* and *Poa*, most of which have arisen by polyploidy, but the extremes of variation in these genera are no greater than they are in many other primarily diploid genera of which the total number of species is much smaller. Since the polyploid members of a complex are more numerous and wide-spread than the diploids, one would naturally expect that as a polyploid complex becomes older and as conditions cease to be favorable for the type of plant represented by that particular complex, its diploid members would be the first to go. An old or senescent polyploid complex, therefore, is one that consists only of polyploids. With increasing age, the polyploids also begin to die out, so that in the last stages of its existence a polyploid complex is simple once more, and is a monotypic or ditypic genus without any close relatives. Examples of such vestigial polyploid complexes, that is, of isolated monotypic or small genera with high chromosome numbers, are scattered throughout the plant king-

dom. Perhaps the most striking ones are the two living genera, *Psilotum* and *Tmesipteris*, which are the only survivors of the most ancient order of vascular plants, the Psilotales. Both of these genera are frequently considered to be monotypic; their species have more than a hundred chromosomes in their sporophytic cells. They may represent the remnants of polyploid complexes which flourished hundreds of millions of years ago in the Paleozoic era. We know from fossil evidence that this order formed a dominant part of the earth's vegetation at that time. Other vestigial polyploid complexes are probably the redwood, *Sequoia sempervirens*, and the genera *Lyonothamnus*, and *Fremontia*, familiar relic species of our California flora which have high chromosome numbers (Stebbins, unpub.). The evidence from the plant kingdom as a whole, therefore, suggests that polyploidy has been most important in developing large, complex and widespread genera; but that in respect to the major lines of evolution, it has been more important in preserving relics of old genera and families than in producing new ones.)

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ON THE QUESTIONABLE EXISTENCE OF SEX CHROMOSOMES IN THE ANGIOSPERMS

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THOUGH the occurrence of sex chromosomes in many species of the Angiosperms has gained almost general acceptance, there is, however, opinion to the contrary. Were we to ignore the physiological aspects of the matter, there would still be two schools of thought among cytologists. Under the pressure of genetics and its arithmetical approach much work has been contributed towards the conception that sex determination depends upon certain types of mechanisms which operate among the chromosomes during the reduction division. With one notable exception, the male plants in the Angiosperms are believed to possess the male and female determining factors and are therefore considered heterozygous to sex. Under normal conditions the pollen of such plants is of two kinds: microspores which carry the male determinant and the other half of the microspores carrying the female determinant. Numerous instances of sex chromosomes have now been described for the Angiosperms. On the other hand, some morphologists have found considerable objection to the above point of view and would include other evidence before letting the question go as a settled fact. This school of opinion is less enthusiastic about arithmetic and more critical of mechanisms than are the cyto-geneticists. Despite the extremely small chance of arriving at any compromise between these two points of view, a review of the literature on sex chromosomes in the higher plants leaves one with the impression that the issues involved in this whole problem are in a fine state of confusion. When, at this late date, the occurrence of a pair of unequal chromosomes during the first division of microsporogenesis is considered sufficient evidence in itself to claim the presence of an X-Y sex chromosome mechanism,

despite the fact that heterochromosomes are common enough among hermaphroditic plants of hybrid origin, then it is perhaps time to make a short but thorough tour of inspection.

Let us begin with a restatement of the conditions upon which the theory of chromosomal sex determination rests. As with other conventional theories on inheritance, the conception of sex chromosomes demands that one should hold rigidly to the idea that a chromosome has constant form, constant character and constant ability to impress its genetical pattern upon the ontogeny of an individual. Except for certain mechanical devices by which a degree of variation is permitted, this constancy must prevail from generation to generation, from division to division. Should the individuality of the sex chromosomes be lost but for one instant somewhere along the line, the idea of sex chromosomes must collapse. This brings us to the question of synapsis, which, in the opinion of most biologists, is no question at all. Nevertheless, it is true that the individuality of the chromosomes is preserved at a crucial stage by the idea of chromosome pairing in anticipation of the reduction division—synapsis or active pairing. The assumption of synapsis is indispensable because it is the only conceivable arithmetical arrangement whereby the reduced number of chromosomes can be derived from the diploid number and still retain the strict individuality of a specific chromosome. Were it demonstrated, for the sake of making the point, that the premeiotic chromosomes do not exist in a free state until late in the prophase, synapsis would come close to being an engineering impossibility. What then of pairing and the dependent individuality of the chromosomes, and what of the chromosome theory of inheritance itself? Under such circumstances the idea of sex chromosomes would be ridiculous. Elaboration of this contention will appear in a later paragraph.

Assuming that all is well with the individuality, the specificity and the synaptic pairing of chromosomes in

general, we must make other conditions. It is necessary to supply a reasonable mode of origin for these sex chromosomes. Since there can hardly be sex chromosomes in a hermaphroditic individual, and since the unisexual types seem to have arisen out of the hermaphroditic state, how then have sex chromosomes developed? Though complete substantiation might not be necessary, at least a rather good hypothesis for their origin would be desirable.

Furthermore, it seems necessary to demand that the phenomena now given credit as being sex chromosomes can not be essentially duplicated among the chromosomes of hermaphroditic organisms. That is, there must be something unique about sex chromosomes, other than purely theoretical imputation.

At least two other things need explanation. The first is the occurrence of intersexes, hermaphrodite or monoecious individuals, among members of an otherwise dioecious species for which sex chromosomes have been described. The second is the reported abnormalities in the actual sex-determining mechanism itself. For example, the female has the smaller number of chromosomes in *Rumex acetosa*, while in *R. acetosella* the reverse is generally true. Furthermore, the mechanisms do not operate with anything near 100 per cent. efficiency. Just what connection do such anomalies have with the organic disposition of the specific plant? For these too one ought to receive a reasonable answer.

Last, but not least, one must be very certain that there is no other explanation of the phenomena now called sex chromosomes which might have as much or more basis in fact. From the genetical point of view it might appear quite logical that there should be such things as sex chromosomes. However, such an argument is not likely to impress any one except those already prejudiced in favor of their occurrence. Is it not possible that the transfer of these genetical values, called factors or units, from the purely mathematical realm to a specific portion of an organic structure (such as a chromosome) may entail a longer jump than is now commonly contemplated?

We have now set before us a list of the principal conditions which, in the opinion of the author, the description of sex chromosomes ought to meet.

1. Individuality and specificity of chromosomes.
2. The continued success of the synaptic theory.
3. A reasonable mode of origin for sex chromosomes.
4. A unique apparatus among all types of chromosomes.
5. A good explanation of intersexes.
6. A good explanation for the frequent misbehavior of the sex chromosome mechanism.
7. Certainty that the facts can not receive a still better interpretation other than calling them sex chromosomes.

Perhaps a few words concerning the mechanical conception of sex determination should be inserted at this time. The ideas about sex chromosomes have undergone considerable change in an effort to meet the criticism of the physiologists and many experimental morphologists. From the early idea of sex being determined by a distinct chromosome which contained a single unit of inheritance for sex, the current opinions allow the interplay of numerous units so that the resulting sexuality of an individual is more of a dominance of one sexual tendency over the other. That is to say, an organism is more female than it is male, and *vice versa*. Though such maneuvering has largely satisfied the earlier criticism, the essential idea of the theory has been maintained, namely, the determination of sex depends in the last analysis upon the distribution, random or otherwise, of certain chromosome structures. No matter how much removed from the original idea of a single unit of sexual inheritance, the theory of sex chromosomes still depends upon a mechanism for its successful operation. Though an opinion on this question when brought down to its simplest terms is likely to be philosophical rather than biological, we face a choice, and our subsequent biological discipline will reflect that choice. We either look upon the chromosomes as the basic substance out of which the manifestations and organic development of life depend or we believe that the chromosomes were among the earlier structural differentiations

of the protoplasm. Many of us have been taught that organic structures have always arisen as the result of some previous action within a still more basic substance. The present theories of chromosome inheritance would largely deny this premise. In fact, they must deny it, since their assumption places the chromosome as the residence of at least the controlling factors if not the basic powers themselves. The chromosome theory of inheritance has developed elaborate schemes to explain the change which takes place in the transfer of inheritance from one chromosome to another. This too is mechanical. Though not essential to the argument of this paper, the analysis of the predicament in which we find ourselves when trying to untangle the logic behind much that has become traditional theory may have its place after all. It seems likely that one is either content or critical of the mechanical conception of organic evolution. Not that being mechanical either adds or detracts from its soundness—but simply that the chromosome theory of inheritance, including sex chromosomes, is based upon a mechanical conception of events and must therefore defend itself upon that foundation.

To examine the chromosome theory of inheritance and its relation to the description of sex chromosomes under each of the seven objective conditions just listed would call for more words and a more secure knowledge of a wealth of material than supposed authorities on the subject have apparently cared to assume. Moreover, the author has delivered himself on several of those points in other papers. The focus of this paper will dwell on points 2 and 7, namely, the continued success of the synaptic theory and the presentation of what the author believes to be a more inclusive explanation of the phenomena now called sex chromosomes.

I. SEX CHROMOSOMES AND SYNAPSIS

Since it is perfectly obvious how essential the synaptic theory is to any consideration of sex chromosomes, the

author has been careful to examine the origin of the meiotic chromosomes in a number of unisexual species for which such sex chromosomes have been described. Without exception, the premeiotic spireme is fundamentally continuous in *Rumex acetosa*, *Rumex acetosella*, *Lychnis alba*, *Smilax glauca*, *Smilax rotundifolia*, *Dioscorea quaternata*, *Ilex opaca*, as well as in numerous other dioecious and perfect species of the Angiosperms (cf. 1936a and 1937). This seems to have been Strasburger's early conception and may still be noticed in several drawings in the later editions of his text-book. Jeffrey (1937) has revived this idea and has lately (Jeffrey and Haertl, 1938) declared his opposition to the synaptic theory of meiosis. The accompanying drawing of premeiotic spireme in a P.M.C. of *Smilax rotundifolia* is fairly typical of similar stages in the above-named plants. This species of *Smilax* in the vicinity of Swannanoa and Black Mountain has $16-n$ chromosomes. If, by conventional theories, there are $32-2n$ chromatids, and each chromatid has two ends (and it can not have less), then each nucleus at this stage

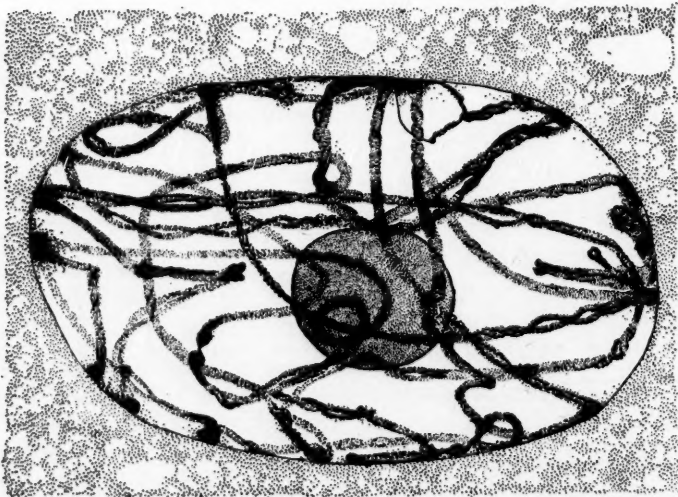


FIG. 1

should have 64 *free ends* visible. The nucleus shown on this page has but one free end visible. Certainly out of a possible 64 at least a dozen free ends would not be too much to ask. But they are simply not there, because the spireme is continuous to the full of its length. The nuclei in the reproductive tissue of *Smilax rotundifolia* are small in comparison with those of *Trillium*, *Allium*, *Lilium*, etc., and consequently not a great deal could be seen as to the spiral composition of the spireme other than it is composed of two principal "threads" whose gyres twist in opposite directions. In a few places along the spireme where its member "threads" are more distinctly separated there is evidence that each "thread" is in turn composed of two "threads," also spiral wound about each other in opposite directions. Where, then, are the individual chromosomes in the diploid number? For the sake of brevity one might advance the notion that each principal thread of the spireme represents sixteen somatic chromosomes joined end to end, and that this thread has "paired" with a similar string of sixteen somatic chromosome mates. For such a claim it is necessary to assume that the individual chromosomes became oriented into the exact position of order in each string and got its right end attached to the correct end of its anterior or posterior neighbor and that when the two strings began to pair the right end sought out the corresponding end of the sister thread. All this is hard to imagine, particularly since the double condition of the spireme is visible almost as soon as any definite structure can be discerned in the nucleus, even at the supposed "leptotene stage." In other words, synapsis in these species approaches an engineering impossibility. Rather does it seem probable that the haploid number of chromosomes becomes segmented out of a fundamentally continuous spireme, the two threads of which originate by splitting of an earlier thread. The separation of the meiotic chromosomes out of the spireme occurs more quickly in some species than in others so that the individual "synaptic pairs" exist some time before diaki-

nesis. On the other hand, particularly in hybrids, the old spireme connections between the haploid chromosomes persist even into the metaphase and anaphase of the heterotypic division (J., 1936a). Sometimes in *Smilax rotundifolia*, the otherwise continuous spireme would show a few breaks in its continuity, but in no instance did the number of *free ends* exceed half a dozen. A word about technique ought to be inserted at this time. The greatest care is necessary in order that the actual conditions of the nuclei may be preserved. Several fluids were used, including Carnoy's, with rapid penetration being insured by the use of a vacuum pump upon immersion. The material was then imbedded according to the Jeffrey (1928) hot nitrocellulose method. In the case of *S. rotundifolia* the use of hydrogen peroxide was not necessary. The sections were left in iron alum for two hours and in Heidenhain's haematoxylin for twenty-four hours. The decoloring process was done very slowly to get the maximum differentiation. In studying these prophases a superficial glance can be wildly misleading. Often the loops of the spireme are so bent that one half of the loop is optically covered by the other half. Several such loops are visible in the drawing. One is first tempted to consider such a loop as a free end of a single pair of chromatids. Nevertheless careful study, in a manner which no photomicrograph can show, will usually reveal that what may appear on first sight to be distinct chromosomes are actually portions of a fundamentally continuous spireme.

Consequent to these studies, the author can not subscribe to the theory of synapsis or active pairing of reproductive chromosomes. The behavior of the premeiotic chromosomes suggests an origin hardly favorable to the present insistence on the individuality of the chromosomes, and, as a consequence, make the support of the sex chromosome idea quite untenable. Few there are who will welcome such a conclusion and yet, if the tremendous possibilities of W. D. Francis's (1939) opinion on "The Spiral Structure of Protoplasm" eventually find applica-

tion in a similar crystalline relationship between cytoplasm and chromatin, then our present ideas on chromosomes are in for considerable modification anyway; all of which may prove very good for cytology.

II. A SUGGESTED EXPLANATION OF THE PHENOMENA GENERALLY CLASSIFIED AS SEX CHROMOSOMES

In an effort to present what the author believes to be a better explanation of the cytological phenomena hitherto called sex chromosomes, it is necessary to introduce further evidence. The first is a brief review of the systematic position of the various Angiosperms for which sex chromosomes have been described. It is quite possible that some papers describing sex chromosomes have escaped the author's attention, and knowledge of such omissions will be appreciated. Though the accompanying compilation may not be complete, the fifty species which are listed undoubtedly include the large majority of plants under discussion and certainly include all the better known or "classical" cases.

A glance at the lists will show that out of forty-nine species and one variety only the two species of *Humulus* belong to a family all members of which are strictly dioecious. However, the system of Engler and Diels was followed in citing the family. Had the system used in Gray's Manual been followed, *Humulus* would be included in the *Urticaceae*. In that case, not one would belong to a family all the members of which are strictly dioecious. Ten out of fifty belong to strictly dioecious sub-families. Twelve belong to strictly dioecious genera. The species of *Salix* and *Populus* can not be included as of strictly dioecious genera, even though they are often thought of as such. As indicated, *Salix Medemii* is strictly a monoecious species according to Engler and Diels (1936). Professor M. L. Fernald, of Harvard University, has advised me that numerous instances have been reported of varying sexual conditions in the flowers of these two genera. Perfect flowers have been noted in *Salix aurita* L., *S. fra-*

gilis, L., *S. amygdaloides* Anderss. and other Old World species. Cases of perfect flowers in *Populus monolifera* and *P. tremula* have been recorded. However, it is not just a case of perfect-flowered intersexes, because many gradations have been observed from perfect, to flowers of both sexes in the same ament, to monoecious and polygamous, as well as the more common dioecious state. Cases where carpels have changed into stamens and vice versa are also well known. It therefore seems obvious that the species of *Salix* and *Populus* are generally in a state of flux and have not established a definitely unisexual condition as yet.

Species 1 to 17 plus Nos. 48 and 49 are characteristic as a group in that they belong to genera containing both hermaphroditic as well as dioecious species (we could also include the species of *Salix*). This would indicate that the unisexual condition of these nineteen species is of particularly recent origin. This group includes the "classical cases" of sex chromosomes ascribed to *Rumex*, *Lychnis* (*Melandrium*) and *Helodea* (*Elodea*). These facts would seem to add up to the opinion that almost all the Angiosperms for which sex chromosomes have been described belong to groups which still show considerable instability as to sexual differentiation and for the most part have only recently acquired the unisexual habit.

It is also of some interest to notice that thirty-nine of the fifty species belong to genera having thirty or more species. Eight out of fifty belong to genera having less than ten species. No species in the list is monotypic. Though the genus *Fragaria* is only given eight species, numerous varieties exist for some of these species, especially *F. virginiana*, some of them being interspecific hybrids.

Though the numerical quantity of forms within a certain genus can not accurately be compared with the number within another genus, since this number depends so often upon the degree to which varieties are given species standing, it is nevertheless obvious that these plants which

are supposed to have sex chromosomes belong to genera of considerable size. Perhaps the diversity of sexual habit and relatively large number of forms found in these genera appear to have little bearing on the discussion. However, these two tendencies may have significance when the cytological condition of these genera is examined more closely. The occurrence of hybrids and varieties has already been mentioned for *Fragaria*. The author's investigation of the genus *Rumex* (J., 1936a) give ample evidence that the extreme polyploidy in the Eulapatham section of the genus is the direct result of interspecific hybridization and, though polyploidy in the Acetosa section has been more restricted, many of the species still possess meiotic irregularities diagnostic of a hybrid ancestry. Various chromosome numbers occur within a single species. For example, *Rumex acetosa* has at various times been found to have 14, 15, 16, 17, 20, 21, 22 and 29 diploid chromosomes. The literature is filled with cases of natural hybrids in this genus. Even with a loose standard, this is hardly a stable condition; yet for *R. acetosa* more effort has gone into the description and study of the sex chromosomes than any other species of higher plants.

The genus *Lychnis* has not given much evidence of polyploidy so far, but it is common knowledge among horticulturists that numerous garden varieties and hybrids exist for several species. The author found that the hermaphroditic *Lychnis chalcidonica* possessed a normal reproductive process, while the dioecious *L. alba*, for which sex chromosomes have been described, presented unmistakable evidence of a hybrid ancestry. The meiotic process displayed lagging chromosomes, chromosome fragmentation, extruded chromatin, occasional extra nuclei and abortive spores. The degree of pollen sterility in some anther sacs was very high. These are characteristic phenomena of known hybrids in contrast with the regular behavior of the parents (J., 1939) and without involving the so-called X-Y apparatus itself bespeak a hybrid ancestry not too distantly removed for *L. alba*.

No.	Name of plant	Authority for sex chromosomes	Family name	Sexual condition of family	Sexual condition of sub-family	Sexual condition of genus	No. of species in genus	Other notes
1.	<i>Bumelia acutosa</i>	Kihara and Ono	Polygonaceae	Herm.—Di.	Herm.—Di.	Herm.—Di.	100	mM complex
2.	<i>B. obtusifolia</i>	Meurman	"	"	"	"	"	"
3.	<i>B. thyriflora</i>	"	"	"	"	"	"	"
4.	<i>B. arifolia</i>	Kihara and Ono	"	"	"	"	"	"
5.	<i>B. nitida</i>	"	"	"	"	"	"	"
6.	<i>B. montana</i>	"	"	"	"	"	"	"
7.	<i>B. hispanica</i>	Jaretsky	"	"	"	"	"	"
8.	<i>Lychinus alba</i>	Blackburn, Winge, Heltz	Caryophyllaceae	Herm.—Di.	Herm.—Di.	Herm.—Di.	90	X-Y complex
9.	<i>L. dioica</i>	Blackburn	"	"	"	"	"	"
10.	<i>L. glauca</i>	"	"	"	"	"	"	"
11.	<i>L. glutinosa</i>	"	"	"	"	"	"	"
12.	<i>L. rubra</i>	Meurman, Heltz	"	"	"	"	"	"
13.	<i>Silene alites</i>	Blackburn	"	"	"	"	"	"
14.	<i>Valeriana dioica</i>	Meurman	Valerianaceae	Herm.—Di.	Herm.—Di.	Herm.—Di.	400	X-Y complex
15.	<i>Fragaria elatior</i>	Kihara	Rosaceae	Herm.—Di.	Herm.—Di.	Herm.—Di.	200 plus	"
16.	<i>Xanthoxylum piperitum</i>	Nakaijima	Rutaceae	Herm.—Di.	Herm.—Di.	Herm.—Di.	15	"
17.	<i>Veltheimia canadensis</i>	Naitos	Hydrocharitaceae	Herm.—Di.	Herm.—Di.	Herm.—Di.	8	"
18.	<i>Veltheimia carolinensis</i>	Winge	"	Herm.—Di.	Herm.—Di.	Herm.—Di.	3	"
19.	<i>Buckleya foena</i>	Nakaijima	Santalaceae	Herm.—Di.	Herm.—Di.	Herm.—Di.	4	"
20.	<i>Urtica dioica</i>	Meurman	Urticaceae	Mon.—Polyg.—Di.	Di.	Polyg.—Di.	30	"
21.	<i>Empetrum nigrum</i>	Hagerup	Empetraceae	Polyg.—Di.	Polyg.—Di.	Polyg.—Di.	3	"
22.	<i>E. hermaphroditum</i>	"	"	"	"	"	300	No s.x. in some sps.
23.	<i>Smilax hederacea</i>	"	"	"	"	"	"	"
24.	<i>S. Oldhami</i>	"	"	"	"	"	"	"
25.	<i>S. China</i>	"	"	"	"	"	600	No s.x. found in <i>D. guatemalensis</i>
26.	<i>D. sinuata</i>	Meurman	Dioscoreaceae	Herm.—Di.	Di.	Di.	30	Numerous instances
27.	<i>D. gracillima</i>	"	"	Mon.—Di.	Mon.—Di.	Di.	"	"
28.	<i>Populus trichocarpa</i>	"	Salicaceae	"	"	"	"	"

No.	Name of plant	Authority for sex chromosomes	Family name	Sexual condition of family	Sexual condition of sub-family	Sexual condition of genus	No. of species in genus	Other notes
29.	<i>P. belamifera</i>	Meurman	Salicaceae	Mon.—Di.	Mon.—Di.	Di.	30	Numerous instances of perfect flowers
30.	<i>P. Simoni</i>	"	"	"	"	"	"	
31.	<i>P. Eugeni</i>	Blackburn	"	"	"	"	"	
32.	<i>P. generosa</i>	"	"	"	"	"	"	
33.	<i>P. serotina</i>	"	"	"	"	"	"	
34.	<i>P. tremuloides</i>	Blackburn and Harrison	"	"	"	"	"	
35.	<i>P. Sieboldii</i>	Nakaijima	"	Mon.—Di.	Mon.—Di.	Mon.—Di.	170	<i>Salix Medemii</i> strictly mon.
36.	<i>Salix Anderssonia</i>	Blackburn and Harrison	"	Mon.—Di.	Mon.—Di.	"	"	
37.	<i>S. reticulata</i>	"	"	"	"	"	"	
38.	<i>S. Bekt</i>	Nakaijima	"	"	"	"	"	
39.	<i>S. Caprea</i>	"	"	"	"	"	"	
40.	<i>S. grandulosa</i>	"	"	"	"	"	"	
41.	<i>S. babylonica</i>	"	"	"	"	"	"	
42.	<i>S. integra</i>	"	"	"	"	"	"	
43.	<i>S. sachalinensis</i>	Kihara, Nakaijima	"	"	"	"	"	
44.	<i>Hamulus lupulus</i>	Winge	Moraceae	Di.	Di.	Di.	2	
45.	<i>H. japonicus</i>	"	"	"	"	"	"	
46.	<i>H. lupulus</i> var. <i>Cordifolius</i>	"	"	"	"	"	"	
47.	<i>Coccolithus trilobus</i>	Nakaijima	Menispermaceae	Di. (almost always)	Di.	Di.	11	
48.	<i>Trichosanthes japonica</i>	"	Cucurbitaceae	Herm.—Mon.—Di.	Mon.—Polyg.—Di.	Mon.—Di.	50	
49.	<i>T. cucumeroides</i>	"	"	Herm.—Di.	Di.	Di.	600	
50.	<i>Dioscorea Tokoro</i>	"	Dioscoreaceae	Herm.—Di.	Di.	Di.	600	

Note. The systematic nomenclature taken from Engler-Diels "Syllabus der Pflanzenfamilien," 11th ed., 1936, Gebrüder Borntraeger, Berlin.
The above table has been compiled from the lists and references given by Galsler (5), Darlington (2), Sharp (15), and Nakaijima (14).

Key to abbreviations
Herm. Hermaphroditic
Mon. Monoecious
Polyg. Polygamous
Di. Dioecious
s.x. Sex chromosomes

In his recent paper Nakajima (1937) reports the presence of laggards among the meiotic chromosomes of the following species, for which he also described sex chromosomes. *Cocculus trilobus*, *Xanthoxylum piperitum* (also the presence of a univalent, small in Nakajima's material, large in Sinoto's material; also premature migration of some chromosomes), *Smilax Oldhami* and *Smilax China*. The author has investigated two native species of *Smilax*, *S. glauca* and *S. rotundifolia* (J., 1937). Meiosis in *S. glauca* takes place without any visible abnormalities in microsporogenesis which could be even vaguely interpreted as heterochromosomes. In *S. rotundifolia* meiotic irregularities do occur in microsporogenesis, just as in most hybrids or species of hybrid origin, but the irregular behavior of certain chromosomes could hardly be allied with sex determination. A numerical difference in the chromosome complements of the four microspores did exist as a result of an abnormal type of reduction division in approximately two thirds of the P.M.C.'s. The remaining third behaved in the normal manner. However, so many other peculiarities were associated with these divisions that it was difficult to conceive of any sex chromosome mechanism having been developed. From the descriptions of meiosis by Nakajima and the author's own investigations it appears that the genus *Smilax* is composed of species of varying cytological disposition; some of which are definitely not far from previous hybridization.

Though most of the six hundred species of *Dioscorea* are found in tropical regions, a few are found further north. Of these *D. quarternata* (Walt) Gmel. was found to possess an odd chromosome which misbehaved in about a third of the P.M.C.'s. It sometimes took on the appearance of a heterochromosome pair, and sometimes split into three fragments. However, there was not sufficient regularity of behavior or the desirable nuclear deficiency to dare consider such a feature a sex chromosome complex. To consider the abnormalities rather as vestiges of

hybrid induced irregularities seemed more compatible with the facts.

Tippo (1938) has gathered the chromosome numbers of the *Moraceae* and presents the following list: 8, 9, 10, 12, 13, 14, 15, 16, and 28. For the *Urticaceae* 7, 12, 13, 14, 16, and 24. According to Gaiser (1930) *Populus* has numbers of 4 and 19, *Salix* 19, 22, 38 and 57. Woodworth (1929) came to the conclusion from his studies on the *Betulaceae* that dysploidy, such as in *Moraceae*, *Urticaceae* and *Salicaceae*, is the result of meiotic irregularities instigated by interspecific hybridization. Therefore the existence of this uneven form of polyploidy, dysploidy, in the three families under discussion is significant in that the abnormalities which gave rise to such odd haploid numbers may have been responsible also for the occurrence of these so-called sex chromosomes.

Consequent to this study of the fifty Angiosperms which are supposed to have sex chromosomes, we might summarize the results, as follows.

The best possible species in which to search for sex chromosomes should have the following characteristics.

1. The species should belong to a large and variable genus.
2. The species should belong to a genus in which both perfect and dioecious species occur.
3. The species should belong to a genus wherein the species display frequent modifications or reversals of the general sexual habit.

Warning! Avoid investigating monotypic species which give no sign of variation.

This summary might be rewritten to read: the phenomena interpreted as sex chromosomes are most likely to appear among species subject to previous hybridization and in which the unisexual habit is of comparatively recent acquisition and as yet not completely stabilized.

The author has been forced to take the position that the plants possessing certain cytological phenomena hitherto called sex chromosomes belong to that innumerable host, plants of hybrid ancestry. Since it is implied that hybridization has been the agent whereby these meiotic irregularities, along with many other types of chromo-

some behavior, have arisen, we needs must answer one specific criticism. It is necessary to explain the constant difference in the chromosome complement of the male and the female plants of a species possessing sex chromosomes. For example, if they are not sex chromosomes, why do the female plants of *Rumex acetosa* have fourteen diploid chromosomes while the male plants have fifteen chromosomes, generation after generation? In this case there is not only a recognizable difference between the supposed male and female determining chromosomes, but a numerical difference in the complements as well. From a morphological point of view this argument is the soundest bulwark in the sex chromosome idea. Here at least there is nothing vague or statistical. Yet there is an answer.

In the first place, the numerical difference is not always constant. Witness the male plants of *Rumex acetosa* with 15, 16, 17, 20, 21, 22 and 29 diploid chromosomes. This variability is also true of the structural difference in the heterochromosomes or sex chromosome complex of *Rumex acetosa*, *R. acetosella* and *Lychnis alba* (J., 1936a and 1936b). Nevertheless, despite any vagaries which might occur among the so-called sex chromosomes, it is evident that there is at least a *constant tendency* toward the maintenance of these visible differences in the chromosomes of the two sexes. For this too there is an explanation built upon the knowledge of hybrid cytology. Let us go back for a moment to the circumstances surrounding the origin of the dioecious condition itself.

Suppose that a certain race of plants, lately hybridized, gradually developed from the hermaphroditic to the unisexual condition. The advent of the unisexual state introduces a new situation in the race. Each act of fertilization actually becomes an act of hybridization, since no two individuals of this hybrid race are likely to have exactly the same genetical composition. Unlike a hermaphroditic race wherein the chances are best for fertilization of an egg by sperm originating from spores of the same plant

as the egg (making proper exception for cases of self-sterility or unusual floral mechanics), the dioecious plant broadens the base for competition for the egg. The sperm must now come from some other plant, and might just as well be from a plant of the same species or some related and compatible variety, species or genus. Thus the unisexual conditions insures the constant introduction of "alien blood." This arrangement also amounts to a postponement of the normal progress of plants of hybrid origin towards the establishment of completely normal meiosis. We can therefore expect to find that such meiotic disturbances which have originated through interspecific hybridization are much more likely to persist in the plants which take on the unisexual habit than in those which remain hermaphroditic or monoecious. The genus *Rumex* exhibits this in a remarkable manner. The *Eulatham* section of the genus is highly polyploid, hermaphroditic and prone to hybridize in nature. Yet the regular species are inclined to be normal in their meiosis (J., 1936a and 1936b). On the other hand, the *Acetosa* section is but irregularly polyploid (dysploid), dioecious and seldom hybridizes in nature. Yet almost all the species of this section which have been investigated display numerous meiotic abnormalities reminiscent of the natural hybrids found among the species of the *Eulatham* section. To the evidence that these plants for which sex chromosomes have been described are of hybrid origin and decidedly unstable as to sexual condition we may now add the conclusion described above that the dioecious condition introduces a circumstance by which this instability is likely to survive for a much longer time than in the hermaphrodite forms.

With the previous paragraph as a background, we may now proceed to a closer scrutiny of the actual relationship thought to exist between the sexes. In conventional terms this is described as being either homozygous or heterozygous to sex. That is, one sex of a given species contains determinants for both sexes of the offspring, and

is therefore heterozygous to sex. The opposite sex, however, is assumed to contain only the determinants for its own sex and is therefore homozygous to sex. This definition may ignore some of the niceties which some might wish added, but probably includes the principal distinctions. With the exception of *Fragaria elatior*, Kihara (1930), the male has been found to be heterozygous to sex in the Angiosperms. It is along with this heterozygosis that the cytological phenomena of sex chromosomes appear; either the numerical or heterochromosome differentiation in relation to sex determination occurs in but one sex. We must remember that from a morphological point of view the XX "pair" which appears in the reduction division of the megaspore mother cell of all these species with the exception of *Fragaria elatior* behaves and looks just like any other "pair" of autosomes. Only by the supposed association of an X with a Y chromosome (nM with M or N with n) does the female sex chromosome or the chromosome for the homozygous sex become unique among the chromosomes of that sex. Consequently, if as in almost all the Angiosperms it is the male which reveals the meiotic peculiarity called heterochromosomes of sex, the remaining problem is simply to suggest a sound reason why this tendency to abnormal meiosis persistently appears in one sex.

The assumption that one sex was strictly the heterozygous one occurred more than a decade before the first sex chromosomes were described. Strasburger (1910) and Correns (1922) explained the inequality in the number of male and female plants in natural populations by assuming that the male was heterozygous and, though the pollen was probably half male and half female determining in nature, there was a competition between the pollen tubes of the two sorts of spores. Thus, a population in which the males outnumbered the female plants in a three to one ratio was due to the stronger or more rapidly growing tubes of the spores carrying the male determinants. For the most part these ideas were carried over into the

description of sex chromosomes. Here is the crux of the whole matter—because the reproductive cells of one sex were demonstrably variable as to sexual disposition (male or female inclined), it has been assumed that the opposite sex was constant in its sexual disposition, or homozygous. The author challenges that contention. Since these plants for which sex chromosomes have been described are definitely in a state of sexual instability and give every evidence of being rife with hybrid abnormalities among the chromosomes as well as in variability of habit, it seems difficult to subscribe to the idea that the female is anywhere near homozygous to sex. In the face of everything we know about the variable condition of hybrids, it seems more logical to assume that in any given species the eggs in any given female can hardly be presumed to have exactly the same disposition towards the sexuality of the offspring which will develop from these same eggs. On the male side the most flagrant partiality is observed. Not only is the sexual potentiality of the various pollen grains thought to differ, but the ability to transmit this difference into a growth or selective factor in the pollen tubes as well. Thus the assumption of a strict heterozygous and homozygous distinction between the two sexes is the same thing as admitting that the factors which determine sex are not subject to the evolutionary laws which we have found to operate in every other phase of hybrid cytology. According to the current opinion, any change in the sexual habit must therefore occur in the heterozygous sex alone, without a contribution from the companion sex. Such an indifference upon the part of the egg in the Angiosperms would be appalling, particularly in view of the evident hybrid ancestry and sexual instability of the plants for which sex chromosomes have been described.

For the sake of completing the proposed hypothesis, let us assume that both sexes are to some extent heterozygous to sex, even though one sex (usually the male in the Angiosperms) carries a greater dominance. In that case no resort need be made to sex chromosomes for an ex-

planation. If, as in most Angiosperms, the appearance of heterochromosomes or any other peculiar type of meiotic apparatus is associated with the male, then the problem resolves itself into getting sperm, possessing this ability, to fertilize or fail to fertilize the egg less disposed or more disposed to femaleness respectively. Let us use *Lychnis alba* to demonstrate this point. In this species the male is thought to be "heterozygous" and consequently it is in this sex that the heterochromosomes are found. The female possesses an XX "pair" at the time of the reduction division; the divisions are regular; the chromosome complements of the megaspores can not be distinguished. Since *L. alba* gives definite evidence of previous hybridization and its systematic proximity to hermaphroditic species suggest the recent origin of the unisexual condition in this species, it is quite probable that the eggs are variously inclined, some being more strongly female than others. Since regular meiosis is associated with the female side, those eggs which are more strongly female in tendency will naturally find a more compatible match in such sperm as have the full and complementing set of chromosomes. On the other hand, such eggs as are weaker in their sexual tendency to femaleness will find the sperm possessing the deficient complements more acceptable. Hence it follows that though sex is definitely linked with peculiar meiotic phenomena in some species of Angiosperms, the occurrence of the same seems to have no bearing whatsoever on the determination of the sex of the offspring. The principal errors which have led to the assumption of sex chromosomes appear to be the lack of appreciation of the role played by hybridization in species evolution, confidence in the implications of the terms "homozygous" and "heterozygous" and lack of familiarity with the behavior of known hybrids. The author is in no way prepared to give an opinion as to just how much variability exists on the female side of these unisexual forms. Experience with hybrids would suggest that it might be somewhat less on the female side than on the

male. On the other hand, the case of *Fragaria elatior*, already mentioned, suggests that in some cases the female might carry the tendency to exceed the male in meiotic abnormalities. Probably intermediate stages exist between the *Lychnis* type and the *Fragaria* type. It is freely admitted that nothing has been stated concerning the reason for the association of these peculiar meiotic abnormalities with a particular sex. Täckholm (1922) discovered a difference in meiotic behavior in microsporogenesis and megasporogenesis of the *Canina* roses, which is also a common feature to an unpredictable extent in most interspecific hybrids of the genus *Rumex* (J., 1936a). Whatever the final interpretation may be, this point has little direct bearing on the problem under discussion.

It has been the thesis of this paper that a consistent explanation of the cytological phenomena generally interpreted as sex chromosomes could be developed which would be more in harmony with the increasing knowledge of the behavior of hybrids. Because the author has already covered some of the objections to the sex chromosome theory in other papers, they were not repeated here. In some respects it might appear that the essence of the views here expressed consist of a reversal of the common conception of events. Whereas the conventional ideas on sex chromosomes terminate with a mechanical device among the reproductive chromosomes which determines the sex of the offspring, the author's opinion holds that the determination of sex, when combined with the forces initiated by previous hybridization and the acquisition of the unisexual habit in a species, will logically produce the transient phenomenon of "sex chromosomes." It therefore follows that "sex chromosomes" have nothing to do with the actual determination of sex—they are simply one of the by-products of organic evolution.

SUMMARY

The following have been presented:

1. An analysis of the conditions upon which the theory of sex chromosomes rests.

2. Evidence against the idea of synapsis.
3. A study of the peculiar systematic and cytological characteristics of the fifty species of Angiosperms for which sex chromosomes have been described.
4. Discussion of the probable fallacy of assuming that any of these fifty species possess a sex which is completely homozygous to sex.
5. An interpretation of the phenomenon now classified as sex chromosomes which is believed to be more in keeping with the facts.

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SHORTER ARTICLES AND DISCUSSION

ADAPTATIVE DIFFERENCES IN THE EGG MEMBRANES OF FROGS

THE influence of temperature in limiting geographical distribution is apparent in many animal groups. One species will be characteristic of a region where the temperature fluctuates around a certain mean, while yet another is found under warmer or colder conditions. To throw light on some aspects of this problem a study is being made of physiological differences among members of the Salientian genus *Rana* that inhabit northeastern North America. These frogs differ in the extent of their northern distribution and, in a given locality in the time at which they spawn. It has previously been shown (Moore, 1938) that marked differences exist in the embryonic temperature tolerance, northern frogs having lower minimal and maximal temperatures for normal development. Furthermore, northern species compensate for the retarding influence of the low temperature of their environment on development, by a rapid rate of growth (Table 1). The present study is concerned with modifications of the jelly envelopes which limit the developing embryos to particular temperature conditions.

TABLE I

Species	Order of breeding	Northern limit	Embryonic temp. range	Hours to stage 20 at 20° C.
<i>R. sylvatica</i>	1	67° 30' N.	2.5-25° C.	72
<i>R. pipiens</i>	2	60° N.	6 -28° C.	95
<i>R. palustris</i>	3	51-55° N.	7 -30° C.	105
<i>R. clamitans</i>	4	50° N.	11 -35° C.	112
<i>R. catesbeiana</i>	5	47° N.	15 - ?° C.	131

THE JELLY MASS OF SPRING BREEDING FROGS

The three species of frogs which breed early in the year, *Rana sylvatica* Le Conte, *Rana pipiens* Schreber and *Rana palustris* Le Conte have a similar spawn. This is in the form of an irregular oval jelly mass which is usually attached below the surface of the pond. The number of eggs varies from 700 to 900 in *Rana sylvatica*, to from 2,000 to 3,000 in *Rana pipiens*. The volume of these masses several hours after fertilization varies from 100 to 150 cc.

This type of jelly mass presents several problems connected with the exchange of gases between the embryos and the environ-

ment. The outermost eggs are separated from the surrounding water merely by the thickness of their own membranes. Those in the center of the mass, however, are some distance from the periphery (as much as two inches), and the amount of oxygen available for their needs is diminished by the respiration of the eggs nearer to the outside. However, at low temperatures the oxygen available to the inner eggs will be greatly increased as:

(1) The surrounding water will contain more dissolved oxygen leading to a greater diffusion of this gas into the egg mass.

(2) The oxygen requirements are diminished by a reduction in temperature, and those embryos near the periphery will not exhaust the available supply of this gas as rapidly as at higher temperatures.

It would seem likely, then, that the oval type of jelly mass characteristic of *Rana sylvatica*, *Rana pipiens* and *Rana palustris* is best suited to cold waters. At temperatures below 15° C. most of the eggs will hatch, although those in the center of the mass may be retarded in their development. Above this temperature the mortality increases regularly. The inability of eggs in a jelly mass to survive at moderately high temperatures is shown in the following experiments.

Eggs of *Rana sylvatica* were collected under natural conditions and brought into the laboratory for immediate use. They were in stage 14 (Pollister and Moore, 1937). The entire experiment was conducted in a room held at 25° C. Although this is supra-maximal for cleavage stages, it is within the normal temperature range for older embryos. Some of the egg masses were placed in aquaria with just enough water to cover them. They were kept in this manner to determine the behavior of entire jelly masses under temperature conditions simulating those in the environment of the summer breeding frogs to be discussed later. Other masses were divided into groups of 2-5 eggs and placed in finger-bowls. These served as a control for rate of development and temperature tolerance. If, as has been postulated, eggs in the entire mass would suffer an oxygen lack, this could be lessened by keeping an atmosphere of oxygen above them. To test this, entire masses were kept in corked 2L. flasks into which oxygen was bubbled four times daily. The results were as follows (Table 2). Eggs in the entire masses were retarded in their rate of development and finally died in stages 17 and 18. Not one tadpole hatched under these conditions. The eggs in groups of 2-5 de-

TABLE II

EXPERIMENT IN WHICH EGGS OF *RANA SYLVATICA* WERE KEPT UNDER DIFFERENT CONDITIONS. TEMPERATURE 24.1-25.0° C.

Number of experiments	Condition of eggs	Atmosphere	Result
10	In entire mass	Air	Eggs die in stage 17-18. None hatch
3	In small groups of 2-5 eggs	Air	Over 90 per cent. hatch as normal tadpoles
6	In entire mass	Oxygen	Over 90 per cent. hatch as normal tadpoles

veloped at a uniform rate and hatched into normal tadpoles. Likewise the entire masses kept under an atmosphere of oxygen hatched. Some of the eggs in the center of these bunches were slightly retarded when compared with those at the periphery, but they eventually hatched. From these experiments we may conclude that the eggs of *Rana sylvatica* (also true for *Rana pipiens* and *Rana palustris*) are not able to survive in this type of jelly mass at 25° C. Furthermore, it seems likely that the failure to survive is due to asphyxiation. Under natural conditions, however, the eggs are not exposed to high temperatures. When *Rana sylvatica* breeds, the water temperature is about 10° C. and mortality is very low. The two additional species with this type of jelly mass breed at a slightly higher temperature; *Rana pipiens* at 12° C. and *Rana palustris* at 15° C. (Wright, 1914). It is now of interest to learn if the latter two species, breeding under warmer conditions but still retaining the compact jelly mass, differ from *Rana sylvatica* in any way that may be considered as an adaptation to the increased temperature.

Such an adaptation has been found in the stage at which these embryos hatch. It will be recalled that the amount of oxygen consumed in frog embryos increases with age (Atlas, 1938). Thus the oxygen deficiency described previously would become greater and greater the longer the embryo remained in the jelly envelopes. When hatching occurs the young tadpole is in direct contact with the surrounding water. *Rana sylvatica* hatches toward the end of stage 20 and in early 21 (Fig. 1). The tadpole at this time has well-branched external gills and is capable of swimming. *Rana pipiens*, breeding under warmer conditions, hatches in stage 18 before the heart has begun to beat, but when muscular contraction is possible. *Rana palustris*, breeding under still warmer conditions, hatches in stage 17. At this time the tail-bud has just appeared, and muscular contraction after mechanical stimu-

lation is not possible. Thus we find a progressive reduction in the time the embryo remains in the jelly envelopes in species breeding under warmer conditions.

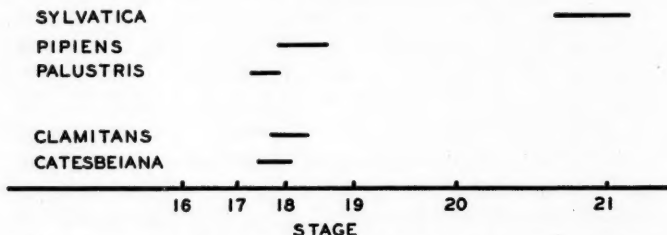


FIG. 1. The morphological stage in which hatching occurs in five members of the genus *Rana*.

THE EGG MEMBRANES OF SUMMER BREEDING FROGS

Rana clamitans Latreille breeds in June when the temperature of pond water has reached 25° C. (Wright, 1914). (We may recall that at this temperature there was no survival in egg mass of *Rana sylvatica*.) *Rana catesbeiana* Shaw breeds in July, and most likely the water temperature is slightly higher. The compact jelly mass so characteristic of the early spring breeding frogs is absent in these two species. The eggs are laid as a film that floats on the surface of the water. Instead of being closely crowded they are well separated, and are connected on but one plane. They are thus in a position to secure a better oxygen supply. An egg film of this nature would be fatal in the early spring breeders, as quite frequently a cold spell after the spawning period causes the surface of ponds to freeze. Eggs so trapped would be killed. Both *Rana clamitans* and *Rana catesbeiana* hatch at an early morphological stage (Fig. 1).

The members of the genus *Rana* employed in this study illustrate a general principle applying to many poikilothermic animal groups; namely, species differ in the temperatures to which they are best suited, as shown by their north-south distribution and their time of breeding in a given locality. In a study of the physiological factors adapting frogs to a particular geographic range and breeding season, it has previously been shown that the embryonic temperature tolerance varies to cover conditions in the environment at the time of spawning, and that northern animals offset the retarding effect of low temperature on development by a

more rapid rate of growth. In the present study it was shown how the structure of the egg mass would tend to limit these species in their breeding. Those frogs breeding early in the spring when the water is cold have a submerged compact jelly mass. The closely crowded eggs, however, die of asphyxiation at temperatures such as those existing in the environment in which *Rana clamitans* and *Rana catesbeiana* breed. The latter two species deposit their eggs in a surface film that insures a better supply of oxygen.

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CROSSING OVER AND SEX IN THE FOWL¹

THE various animals investigated have shown wide divergence regarding the influence of sex on the percentage of crossing over between genes known to be carried by the same chromosome. The extremes of reaction are shown by *Drosophila* on the one hand with crossing over normally occurring only in the female, and the silkworm on the other, with crossing over limited to the male. Other forms show the incidence of crossovers to be practically identical in the two sexes.

The fowl is of special interest, since it differs from most animals in which the phenomenon of crossing over has been investigated, in that the female is the heterogametic sex. It is only recently that sufficient data on favorable material have been accumulated to give evidence of the relationship of crossing over to sex in the fowl. Landauer (1933) and Taylor (1934) have presented a large amount of data on the linkage relations of rose comb and creeper characters in the fowl. Their results involved crossing over in both males and females, but the crossover per-

¹ Contribution No. 117 from the Department of Poultry Husbandry.

centage was so low (less than 1 per cent.) that the data were not favorable for determining the effect of sex on the incidence of crossing over. Considerable data have been published on the linkage relation of sex-linked factors in the fowl, but such data are of no value for establishing the relationship of sex to the phenomenon of crossing over.

TABLE I
EFFECT OF SEX ON CROSSING OVER IN THE FOWL

	Crossing over in females			Crossing over in males		
	Total gametes	No. cross overs	Per cent. crossing over	Total gametes	No. cross overs	Per cent. crossing over
Crest—Dominant White						
Warren	1,268	160	12.3	2,278	280	12.3
Hutt	35	2	5.7	379	55	14.5
Total	1,303	162	12.4 \pm 0.62	2,657	335	12.6 \pm 0.43
Frizzling—Dominant White						
Warren	315	62	19.7	1,222	224	18.3
Hutt	248	46	18.5	337	48	14.2
Total	563	108	19.2 \pm 1.12	1,559	272	17.4 \pm 0.64
Frizzling—Crest						
Warren	584	173	29.6	1,087	301	27.7
Hutt	35	11	31.4	80	24	30.0
Suttle & Slpe	235	68	28.9	81	22	27.2
Total	854	252	29.5 \pm 1.05	1,248	347	27.8 \pm 0.85
Silkie—Flightless						
Warren	1,079	125	11.6 \pm 0.66	580	71	12.2 \pm 0.92
Rose Comb—Creepers						
Landauer	4,313	23	0.53 \pm 0.074	3,095	5	0.16 \pm 0.048
Taylor	683	4	0.59 \pm 0.197	1,500	6	0.40 \pm 0.109
Total	4,996	27	0.54 \pm 0.070	4,595	11	0.24 \pm 0.049

In Table 1 are data on autosomal linkage in the fowl. Both the published data of various workers and previously unpublished results of the author are included. The data from all sources have been combined for the comparison of the crossover percentages in the two sexes and involve 5 pairs of characters. Both the coupling and repulsion phases are included in the totals given for each pair of characters. A very large percentage of the results is from one-year-old birds. The data credited to Hutt are those reported by Warren and Hutt (1936); and those of the author include those given in that publication combined with a considerable body of recently acquired data.

The data in Table 1 show very similar percentages of crossing over in males and females. In no instance is the difference between the sexes more than 2 per cent. The numbers involved are fairly large, and in only one case (rose comb—creepers) is the difference between males and females statistically significant as indicated by the probable errors of the means. In the five tests

reported the females twice slightly exceeded the males in percentage of crossing over, while in the other three, the males slightly exceeded the females. It would seem, then, that the phenomenon of crossing over is very little, if at all, influenced by sex in the fowl. Any existing effect of sex is so slight as to be negligible in the determination of linkage relations in this form.

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ANOTHER NEW YORK RECORD FOR THE FRESH-WATER MEDUSA

THE fresh-water medusa, *Craspedacusta ryderi*, generally believed to be rare, is reported from a few new localities each year. I should like to report its occurrence in Garnet Lake, Warren County, New York. The medusae were seen swimming in the lake on September 7 and 8, 1938. On September 9 about one hundred specimens were taken and preserved. This number was easily taken, for, as other collectors have found, the medusae often occur by the thousands. The medusae, never before reported from this lake, were found in two areas, separated from each other by over a mile of open water.

Garnet Lake is located about fifteen miles south of North Creek, N. Y. On some maps it is also listed as Mill Creek-Pond or Mill Pond. However, the pond was greatly enlarged in 1851 by the construction of a dam across the outlet, and since then it has usually been called Garnet Lake. It is now about two miles long and varies in width from one fourth to one half of a mile. About one half of the lake is very shallow and is filled with decaying tree trunks, which were inundated when the dam was built. The medusae, however, were all found in the open end of the lake.

All the Garnet Lake medusae examined were females. Their ovaries were filled with eggs which were apparently in late stages of oogenesis. Dr. Dayton Stoner, of the New York State Museum, very kindly sent me some medusae which he collected during the summer of 1937 at Loudonville, N. Y.⁴ When gonads of these were sectioned it was found that they were all males still in active stages of spermatogenesis. Spermatozoa were plentiful in all gonads, as were spermatids and groups of dividing spermatocytes. A comparison of the gross morphology of these cytologically determined males with cytologically determined females showed that the sexes are similar in all characteristics except the shape of the gonads. The female gonad is roughly oval or bean-shaped. The male gonad is of the same general shape, except that the distal end is greatly elongated and, therefore, hangs farther down into the sub-umbrella cavity than does the female gonad.

The distinction between the American species of the fresh-water medusa and the European species is not as yet very clear. Although elucidation of all the peculiarities of the two forms must await a complete study of the life histories of both species, the following observation may prove of interest. A comparison of the Garnet Lake medusae with topotypes of *Craspedacusta sowerbii*, loaned to me by the British Museum of Natural History, showed these two forms to be essentially similar except for the size of the tentacles. It was found that tentacles of the same length were much more slender in *C. sowerbii* than in *C. ryderi*. This difference does not seem to me to have resulted from the methods of preservation.

Craspedacusta has previously been reported for New York State by Breder from the New York Aquarium;¹ by Robertson from Lackawanna;² by Davis from Staten Island;³ and by Stoner from Loudonville.⁴

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¹ *Science*, 67: 242, 1928.

² *Science*, 80: 403, 1934.

³ *Proc. Staten Is. Inst.*, 8: 15-16.

⁴ *Science*, 87: 188-189, 1938.

